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Study of baroreceptor sensitivity index in chronic fatigue syndrome

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ABSTRACT

STUDY OF BARORECEPTOR SENSITIVITY INDEX IN CHRONIC FATIGUE SYNDROME

by

Diane L. Donnelly

Although numerous studies evaluate baroreflex response as part of their research protocol, only one was identified as having evaluated continuous baroreceptor response as part of a 70^0 head-up tilt. It was done by Youde et al. [28] and was limited in that it evaluated only sequences of three beats for lag 0. The limitations of the Youde study [28] are not present in this thesis.

The data for this thesis came from a 70^0 head-up tilt study performed on chronic fatigue syndrome subjects by LaManca et al. [3] at the East Orange VA Medical Center. Baroreflex was not evaluated as part of the LaManca study. The goal of this thesis was to determine the utility of using the baroreflex sensitivity index (BRSI) on chronic fatigue syndrome subjects as a marker to discriminate between subject populations. Five groups are studied; five chronic fatigue syndrome (CFS), five chronic fatigue syndrome with postural orthostatic tachycardia syndrome (CFS-POTS), five chronic fatigue syndrome with fibromyalgia (CFS-FM), five control with postural orthostatic tachycardia syndrome (CON-POTS) and five control (CON).

The sequence method was used to assess baroreflex function. Comparative time analyses were done of weighted BRSI values between groups as well as between lags within groups. Baroreflex effectiveness index, BEI, was also evaluated along with the total number of ramps and sequences within and between groups.

Comparative time analysis provided a graphical representation of the behavior of the baroreflex during head-up tilt. Spikes in BRSI in the transitions between baseline and tilt and again between tilt and recovery were most noticeable for CFS-FM followed by CON-POTS and CFS-POTS. The CFS and CON groups were surprisingly similar in their behavior throughout the tilt. Differences between CON and CFS became graphically more apparent when evaluating BRSI by lag and calculating the percent change in sequences by lag. Slope of the weighted average for BRSI was calculated over a moving window of two-minute intervals. Based on this graph, slope does not appear to indicate a large difference between subject populations.

Evaluation of the total number of ramps and sequences by group for each lag indicated that the most efficient use of ramps was seen in lag 0 for all groups. The number of sequences tapered off dramatically in lags 1 and 2 regardless of the availability of ramps. The percent decrease in the number of sequences between lags for each group was calculated. The two most consistent groups in the number of sequences generated from the available ramps across all lags are CON and CFS-FM. The least consistent group is CFS.

The utilization of comparative time analysis to evaluate BRSI is an unexplored area of baroreflex research. Based on the graphical results of this thesis the idea appears to have merit.

STUDY OF BARORECEPTOR SENSITIVITY INDEX IN CHRONIC FATIGUE SYNDROME

by

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STUDY OF BARORECEPTOR SENSITIVITY INDEX IN CHRONIC FATIGUE SYNDROME

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This thesis is dedicated to my parents,
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CHAPTER 1

INTRODUCTION

Baroreflex sensitivity is widely used in scientific research as an indicator of autonomic nervous system activity. The sole purpose of the baroreflex is to maintain arterial blood pressure. When arterial baroreceptors are stretched, signals are sent to the brain causing blood pressure to rise or fall as necessary. Although numerous studies evaluate baroreflex response as part of their research protocol, only one was found that evaluated continuous baroreceptor response. This study done by Youde et al. [28] evaluated continuous baroreceptor measurement in healthy elderly individuals. Each subject, on two separate occasions, underwent three 70^0 head-up tilts that lasted for 10 minutes each. Further, a standardized sequence length of three beats was used and only lag 0 BRS was evaluated. The goal of the Youde study was to test a new technique of mathematical modeling for the assessment of BRS. In order to validate their model they also calculated BRS using spectral or frequency domain analysis. The results from both evaluations were comparable.

For this thesis, head-up tilt was also 70^0, however it lasted for 45 minutes unless presyncopal or syncopal symptoms were exhibited by the subject. All data was gathered in one visit, sequences were considered as three or more beats and lags 0, 1 and 2 are evaluated. Evaluation of baroreflex function was assessed using the sequence method, which produces a baroreflex sensitivity index, BRSI. BRSI is an index that provides an instantaneous indication of the ability of the baroreceptors to control heart rate. The sequence method and BRSI are discussed in detail in Section 1.4. In addition, the

disease state CFS was compared to matched controls to access the utility of a comparative time analysis of BRSI in distinguishing between subject groups.

The data for this thesis was acquired from a study done by LaManca et al. [3]. The LaManca study was done at the East Orange VA Medical Center. This was a 70^0 head-up tilt study performed with the primary focus of determining the diagnostic value of head-up tilt, HUT, in chronic fatigue syndrome, CFS. A secondary goal of the study was to determine how HUT could aid in understanding the cardiovascular autonomic function in CFS [3]. To address these questions thoracic impedance cardiography in conjunction with continuous electrocardiogram, ECG, and blood pressure readings were taken. One of their findings was that the incidence of neurally mediated hypotension was no greater in CFS subjects than in matched controls when comparing positive head-up tilt results. LaManca defines a positive head-up tilt test as one that was stopped prematurely due to presyncopal or syncopal symptoms exhibited by the subject.

It is the goal of this paper to evaluate the effect of the baroreflex in a subset of the LaManca study patient sample to determine whether the baroreflex, analyzed over time, indicates a significant difference between chronic fatigue syndrome patients and sedentary matched controls during positive head-up tilt.

1.1 The Heart

The heart is separated into a left and a right side by a tissue wall called a septum, which is again divided into left and right atria and ventricles by atrioventricular (AV) valves creating a four-chamber organ. The right AV valve or tricuspid valve is located between the right atrium and ventricle. The left AV valve or the mitral valve is located between

the left atrium and ventricle. The left atrium receives oxygen-enriched blood from the pulmonary veins and empties into the left ventricle. The right atrium receives oxygen-deficient blood from the systemic veins, which empties into the right ventricle. There are no inlet valves from the venous systems delivering blood to the atria. The left ventricle delivers oxygen-rich blood to the body through the aortic valve. The right ventricle delivers oxygen-deficient blood to the lungs via the pulmonary valve. In a healthy heart, all valves allow blood flow in one direction only and are opened and closed by pressure gradients.

Contraction of the cardiac muscle is the result of self-generated action potentials by specialized groupings of cells that make up about one percent of the total cardiac muscle. Groupings of these specialized cells or nodes, which are noncontractile, are found in specific locations of the heart muscle. Although each group of cells is capable of stimulating the heart, each has a different rate at which they depolarize; therefore, the fastest operating group drives the heart.

The sinoatrial (SA) node located in the right atrial wall is the main pacemaker because it is the fastest generator of action potentials per minute in a healthy heart [1, 18]. The atrioventricular (AV) node located at the base of the right atrium is the second fastest generator of action potentials and the next in line to take over if the SA node malfunctions. The next section of the cardiac conduction system is the bundle of His, also known as the atrioventricular bundle, which originates at the AV node, separates into left and right bundle branches and spreads through the septum and ventricular walls. The Purkinje fibers are small branches extending from the left and right bundle branches.

Regardless of where the action potential originates, it spreads through the heart muscle in a coordinated manner in a normal heart. The conduction system is shown in Figure 1.1.

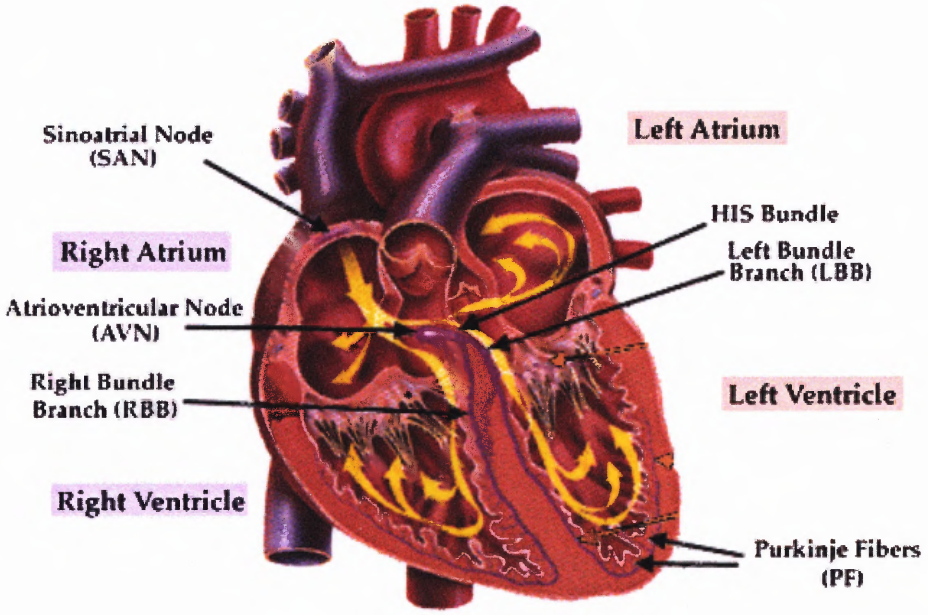


Figure 1.1 Conduction system of the heart. Illustrating the SA node or main pacemaker, the AV node, the Bundle of His, left and right bundle branches and the Purkinje Fibers [23].

1.2 Blood Pressure

Blood pressure, defined as the pressure exerted by the blood against the interior of the vessel wall, is dependent on the volume and compliance of the vessel. During a cardiac cycle, there are two phases of blood pressure, systole and diastole. Systolic blood pressure (SBP) represents approximately one third of the cardiac cycle while diastolic blood pressure (DBP) accounts for the remaining two thirds [1]. Figure 1.2 illustrates blood flow and heart valve operation during both diastolic and systolic cycles. During diastole, the heart is at rest and the atria are filling. The AV valves, opened during much

of this cycle, allow partial filling of the ventricles. When the action potential reaches the AV node, a propagation delay of approximately 100 ms occurs enabling the atria to completely depolarize and contract, emptying their contents into the ventricles. The pressure difference between atrial and ventricular pressure closes the AV valves.

Systole begins as the ventricles continue to depolarize and build pressure until aortic and pulmonary arterial pressures are overcome, at which time they contract, forcing blood into the lungs and the aorta. During ventricular contraction, as ventricular pressure decreases, the aortic valve closes causing a disturbance in the blood pressure waveform known as the dicrotic notch, which is shown in Figure 1.3.

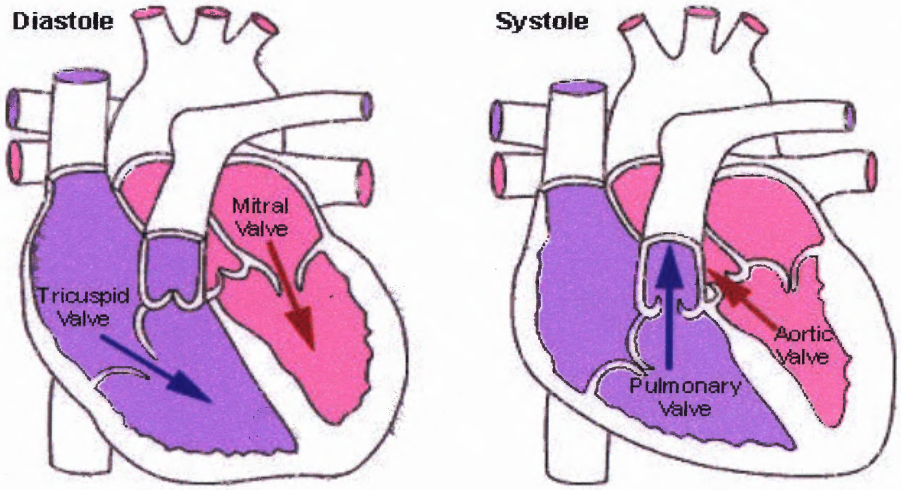


Figure 1.2 Diastolic and Systolic cycles of the heart [24].

The amount of blood pumped out of the ventricles during each cycle is the stroke volume, which is the difference in the ventricular volume before and after contraction. Repolarization of the atria occurs as the ventricles depolarize. Therefore, the atria are in diastole during ventricular systole [1]. A typical blood pressure waveform is shown in Figure 1.3.

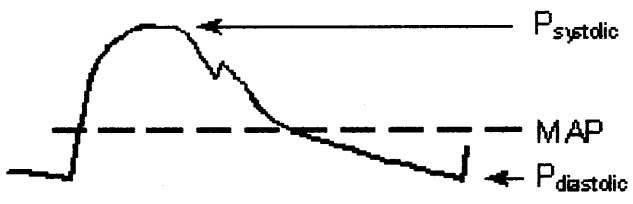


Figure 1.3 Blood pressure waveform illustrating systolic and diastolic levels. MAP is mean arterial pressure [32].

Figure 1.3 is the mechanical waveform caused by the pumping action of the heart as previously described. The notch below $P_{systolic}$ is the dicrotic notch representing a disturbance in the waveform caused by closing of the aortic valve following ventricular contraction [1]. Use of a blood pressure cuff or sphygmomanometer and stethoscope are described in Section 1.2.1. The dotted line labeled MAP indicates mean arterial pressure. Pulse pressure is the difference between systolic and diastolic pressures. MAP can be approximated through use of the formula indicated below in equation (1.1) [32].

$$MAP \cong P_{sys} + \frac{1}{3}\left(P_{sys} - P_{dias}\right) \qquad (1.1)$$

1.2.1 Measurement

Measurement of blood pressure reflects the total force per unit area that the blood exerts on the interior of a blood vessel. Perhaps the most familiar non-invasive blood pressure measurement uses an inflatable cuff attached to a pressure gauge, as shown in Figure 1.4, and is called a sphygmomanometer.

Figure 1.4 Sphygmomanometer [25].

Blood pressure, measured in millimeters of mercury (mm Hg), is usually noted as systolic blood pressure over diastolic blood pressure. The inflatable cuff is placed on the arm above the elbow at about heart level. The cuff is inflated to a pressure above systolic, generally greater than 180 mm Hg. This pressure occludes blood flow to the forearm by compressing the brachial artery. A stethoscope is placed below the cuff in the bend of the elbow to confirm absence of sound [1]. Once occlusion is confirmed, cuff pressure is slowly decreased at an optimal rate of 3 mm Hg per second [1, 2]. The first sound detected by the stethoscope occurs when blood pressure matches the cuff pressure and blood begins spurting through the now only partially occluded artery. The pressure indication on the gauge is systolic pressure. Deflation of the cuff continues until the sound tapers off to a muffle just before silence. At this point, the brachial artery is no longer occluded and diastolic pressure is recorded. Diastolic pressure is indicative of a non-turbulent or laminar flow of blood due to the resting state of the heart. This technique requires a trained ear and a relatively quiet environment. Many factors affect

blood pressure readings such as disease, emotional and physical conditioning, diet and stress. Any or all of these factors among others can and do affect blood pressure readings.

In clinical studies, the use of a sphygmomanometer is not the optimal choice when continuous blood pressure readings are required. One reason is the continual inflation and deflation of the arm cuff would be distracting and uncomfortable for a subject. A better method is an automated blood pressure monitor called the Finapres™ made by Ohmeda.

Finapres™ is an acronym for FINger Arterial PRESure. The Finapres™ uses an inflatable finger cuff with a built-in photoelectric plethysmograph [4]. A plethysmograph is a device used to measure and record changes in volume of the body or a body part [2]. The Finapres™ uses an LED that passes light through the tissue bed of the finger. The transmitted light, picked up by the photo sensor, adjusts finger cuff pressure accordingly to maintain a constant volume. With greater pressures, there is less blood to transmit light; the reverse is true with less pressure.

It is important, when using the Finapres™ to maintain the arm used in measurement at heart level to avoid errors in the blood pressure readings. The LaManca tilt study [3] utilized an adjustable sling that held the subject's arm at approximately heart level during recordings. If this is not practical, 2 mm Hg should be subtracted from the reading for every inch the arm is below heart level or added for every inch that the arm is above heart level [29].

The Finapres™ recording begins by identifying a set point, which is the mean arterial pressure. Initially pressure is applied above systolic pressure to occlude blood

flow. As cuff pressure is reduced, arterial blood flow begins causing oscillations in the cuff. The photo sensor picks up these oscillations. Oscillations increase as the cuff pressure is reduced, eventually reaching a maximum before tapering off as the cuff pressure is reduced to zero. The point of maximum oscillation is true mean arterial pressure [2]. Mean arterial pressure is closer to diastolic than systolic due to the length of the diastolic portion of the pressure waveform in each cycle.

Once mean arterial pressure is identified, the Finapres™ uses this value as a set point for a servomotor which manipulates cuff pressure with the goal of maintaining the amount of light passing through the finger. The pressure changes necessary to maintain the set point mimic the arterial blood pressure waveform, and provide a continuous non-invasive blood pressure measurement. The Finapres™ is also equipped with an automatic calibration circuit that operates once every 70 beats or approximately once every minute [4]. During studies requiring continual blood pressure readings, the calibration circuit is manually disabled.

Continuous blood pressure readings from the LaManca [3] study were collected using a Finapres™ unit. Unfortunately, calibration was not consistently disabled when necessary, resulting in gaps in the blood pressure readings. In order to deal with these problems algorithms were developed to interpolate the systolic blood pressure data to maintain the integrity of the time line. The algorithms developed and the interpolation processes are discussed in Section 2.2.

1.2.2 Blood Pressure Regulation

The vascular tree consists of arteries, arterioles, capillaries, venules and veins. Figure 1.5 is a graphic of the vascular system. The heart pumps oxygen-rich blood into the aorta from the left ventricle and receives oxygen-depleted blood from the vena cava into the left atrium.

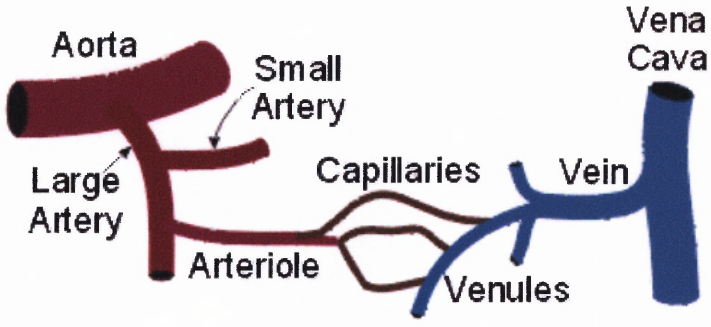


Figure 1.5 Vascular tree [32].

Arteries are the largest in diameter and deliver blood to various organs via arterioles. Arterioles have a much smaller diameter than arteries and thus they present a much larger resistance to the flow of blood. Capillaries receive blood from arterioles. Although they are smaller in diameter than arterioles, they do not present much resistance, due to their parallel configuration. Venules and veins return oxygen depleted blood to the heart. Due to the considerable difference in resistance between arteries and arterioles, the arteries must be compliant enough to hold blood that will not initially flow into the higher resistance arterioles which sets up a pressure gradient in the tissue bed. The ability of the arteries to hold blood allows for continued blood flow during diastole when the heart is at rest and not pumping. During diastole, the arteries relax because no blood is being pumped. As a result, blood flow continues into the arterioles from the

arteries. This pressure gradient is also responsible for systolic to diastolic pressure changes in the arteries [1].

Arterioles are not as compliant as arteries, but they do have a much thicker layer of smooth muscle that is innervated by the sympathetic nervous system, a division of the autonomic nervous system. Arteriole smooth muscle is normally in a partially constricted state known as tone. Tone is due in part to the release of norepinephrine by the sympathetic nervous system [1]. Tone is important as it provides a regulatory mechanism for vasodilation or vasoconstriction based on the needs of the tissue bed. Without tone, it would be impossible to dilate the vessels; regulation would be limited to vasoconstriction.

Mean arterial blood pressure (MAP) is highly regulated by changes in parameters such as heart rate and peripheral resistance. The main control center for blood pressure regulation is the cardiovascular control center (CCC), which is located in the medulla within the brain stem [1]. As previously mentioned arterioles are innervated by the sympathetic nervous system (SNS) which varies constriction or dilation as required by the tissue bed. The parasympathetic nervous system (PNS), another division of the autonomic nervous system, does not innervate arterioles but does innervate the atria of the heart. The SNS innervates the entire heart.

Sympathetic and parasympathetic nervous system activity work in opposition to one another in the heart. When the SNS activates to speed up heart rate, the PNS works to slow it down. The cardiovascular control center regulates the ratio of PNS to SNS activity ultimately controlling blood pressure regulation.

The baroreceptor is a circulatory system sensor whose sole responsibility is regulation of arterial blood pressure within a narrow range [1, 32]. Arterial baroreceptors are stretch receptors located in the carotid sinus and the aortic arch as illustrated in Figure 1.6.

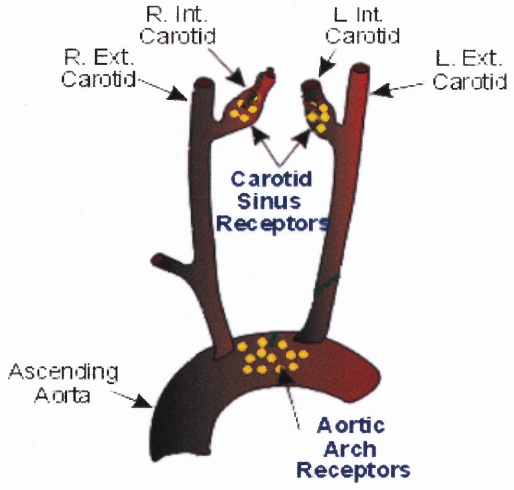


Figure 1.6 Carotid sinus and Aortic Arch baroreceptor location [26].

Baroreceptors are responsive to mean arterial and pulse pressures due to stretching of the arterial walls. Baroreceptors send tonic signals to the cardiovascular control center. Signals from the carotid sinus are relayed to the CCC by cranial nerve IX, the glossopharyngeal nerve. Signals to the CCC from the aortic arch are transmitted by cranial nerve X, the vagus nerve. When stretched, the tonic signal changes relaying new information to the CCC, which responds by changing the PNS and SNS activity to control blood pressure. There are other influences affecting blood pressure regulation. This thesis however, focuses on arterial baroreceptor responses to changes in arterial blood pressure.

1.3 Disease States

As mentioned in the Introduction of this thesis the data was obtained from the LaManca study [3], which was performed at the East Orange VA Medical Center. The chronic fatigue syndrome subjects from the LaManca study were also afflicted with additional diseases. As a result, the disease states examined in the thesis are chronic fatigue syndrome (CFS), fibromyalgia (FM) and postural orthostatic tachycardia syndrome (POTS). A brief discussion of the symptoms of each as well as a discussion of the medical term syncope is presented.

1.3.1 Syncope

The medical term for fainting is syncope. Fainting is a brief sudden loss of consciousness from which there is a complete recovery. One of the causes of syncope is orthostatic intolerance. Orthostatic intolerance manifests itself as the inability to sustain consciousness during standing or a feeling of lightheadedness when standing with the potential of fainting. Symptoms are dizziness and disabling fatigue and may be accompanied by nausea. Syncopal episodes may occur when an individual is standing or changes position from supine to upright, when eating, showering or engaging in light exercise. These activities would have no effect on a normal subject.

When a person stands, blood can accumulate or pool in the lower extremities due to the positional change and the force of gravity [21]. In healthy individuals, the venous system at rest is in a partially constricted state called venous tone due in part to the release of norepinephrine from the SNS [1]. With lower extremity pooling, the SNS is activated thereby constricting the veins and forcing a redistribution of blood. Skeletal

muscles also tense or tighten at this time aiding in blood redistribution. Activity of the SNS through vasoconstriction cannot completely compensate for the effects of gravity without the aid of skeletal muscle [1]. It is very important that the sympathetic nervous system operate properly in conjunction with some degree of muscular tone to avoid syncopal episodes.

In normal healthy individuals, adequate venous and muscle tone generally exists, so during normal activity syncopal episodes are rare. If problems exist, due to diseased states or poor physical condition, compensation for blood pooling may be incomplete or impossible, depriving the brain of a sufficient blood supply and syncope or presyncopal symptoms occur.

1.3.2 Chronic Fatigue Syndrome

Chronic fatigue syndrome (CFS) is clinically defined yet lacks a definable medical cause [3, 7, 30]. Clinically CFS is characterized by debilitating fatigue of more than six months duration as well as a marked decrease in daily activity [30]. Other symptoms may include cognitive impairment, mental fatigue, muscle pain, multi-joint pain, headaches, sleep disturbance, mild fever, swollen or tender lymph nodes, weight loss or gain and sore throat [5, 7]. Because the symptoms of CFS are self-reported, a diagnosis of CFS can only be made after alternate medical and psychiatric causes have been eliminated [30]. For example, anxiety disorders and depression can be accompanied by severe fatigue. Because so many illnesses are associated with fatigue, many CFS patients are often misdiagnosed or diagnosis takes a long time [30].

Many CFS subjects also suffer from orthostatic intolerance (OI) which manifests itself either as the inability to sustain consciousness during standing or a feeling of lightheadedness when standing with the potential of fainting. Some investigators have found a significant overlap in symptoms between orthostatic intolerance and CFS in some CFS subjects; however, the degree to which this overlap exists has not yet been determined [5].

The head-up tilt study by LaManca et al [3] as described in the Introduction showed that by carefully matching healthy but sedentary controls to CFS subjects there exists no significant difference between these two groups in orthostatic intolerance to a head-up tilt test. Thus, it is their finding that a head-up tilt test does not adequately differentiate between chronic fatigue syndrome subjects and normal but inactive adults.

1.3.3 Fibromyalgia

Fibromyalgia (FM) is a musculoskeletal pain disorder that shares some characteristics with chronic fatigue syndrome [8]. Pain in the muscles, ligaments and tendons, and the fibrous tissues in the body define fibromyalgia. The 1990 American College of Rheumatology proposed guidelines for diagnosing fibromyalgia that includes widespread pain of three months or more and the physical finding of eleven or more of eighteen specified tender points [10]. Some secondary symptoms of FM are mild fever, sore throat, lymph node pain, widespread muscle pain and tenderness, joint pain and sleep disturbance. Patients with FM have an overall reduced pain threshold. The principal symptom of FM is pain whereas the principal symptom of CFS is fatigue. However, each share similar secondary symptoms.

1.3.4 Postural Orthostatic Tachycardia Syndrome

Postural orthostatic tachycardia is a syndrome of orthostatic intolerance with excessive tachycardia upon standing or onset of upright posture. Tachycardia is an excessively rapid heart rate generally greater than 100 beats per minute. To receive a diagnosis of POTS a patient must have an orthostatic or standing increase in heart rate of at least 30 beats per minute, and an absolute orthostatic or standing heart rate of at least 120 beats per minute, which is accompanied by symptoms of dizziness, light-headedness, visual blurring, palpitations and weakness [6]. One physiological concomitant of POTS is intrinsic sinus node abnormality, which was noted in one study as being significantly impaired in about 20% of the patient group [6].

When heart rate increases, the PR interval should shorten. Figure 1.7 illustrates a typical ECG and the PR interval. The PR interval represents the AV nodal delay of

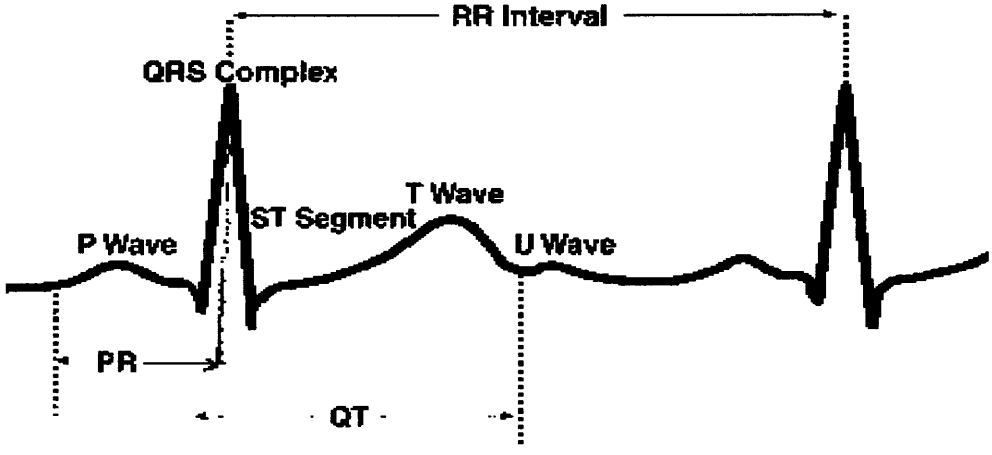


Figure 1.7 Illustration of ECG waveform [27].

100ms, the continued depolarization of the atria and the start of ventricular depolarization. When seated POTS patients have a higher heart rate than controls but the

PR interval differences are not statistically significant between the two groups [6]. With an increased heart rate, the PR interval decrease was significantly less in POTS patients then in controls [6]. This means that even though the heart rate had increased, the PR interval in POTS patients did not significantly decrease, which resulted in a longer than expected PR interval at an increased heart rate. This was an important observation because at baseline there was no significant difference between POTS patients and controls. Although some studies have identified physiologic abnormalities related to POTS overall, the underlying pathophysiology is poorly understood. Moreover, POTS can occur in individuals who appear relatively normal and is likely under-diagnosed [9].

1.4 Baroreflex Sensitivity Index (BRSI)

Baroreflex sensitivity (BRS) measurement is a widely used technique to assess autonomic activity [11,14]. Arterial baroreflex function and specifically baroreflex control of heart rate, is widely used in both cardiovascular research as well as in clinical cardiology [11]. A number of techniques to obtain a dynamic estimate of the gain of baroreflex control of heart rate, called baroreflex sensitivity index or BRSI are currently used in research. This thesis discusses a measure of this gain derived from spontaneously occurring changes in blood pressure called the baroreflex sensitivity index or BRSI through use of the sequence technique. The sequence technique uses systolic blood pressure as the input or independent variable and the R-R interval as the reflexive response or dependent variable. The sequence method is an estimation of BRS that uses linear regression of spontaneous sequences of three or more beats where changes in systolic blood pressure and the associated R-to-R intervals change in the same direction.

The slope of the regression line between these values provides an index of the estimate of baroreflex sensitivity (BRSI) during the specified period.

1.4.1 BRSI Measurement

Assessing the characteristics of baroreflex functionality has become the focus of many studies since research findings have noted that alterations in baroreflex sensitivity may have a diagnostic value for some diseases such as myocardial infarction and heart failure [19,20,21]. Traditional laboratory techniques used to assess BRS in humans were invasive. Injection of vasoactive drugs and the use of neck chambers to manipulate baroreceptors were commonly used invasive techniques [12, 15, 19, 21, 22, 32]. Early laboratory techniques were pivotal to understanding baroreflex function thus providing the background and validation for the development of newer non-invasive techniques. Evaluation of the baroreceptor response before and after surgical removal of baroreceptors in cats demonstrated that spontaneous systolic blood pressure changes could be used to evaluate baroreceptor response [13, 33]. Developments based on non-invasive measures of blood pressure and heart rate have been used to perform computer analyses of these signals. These new non-invasive techniques allow the researcher to understand baroreflex functionality in and out of the laboratory providing a better understanding of baroreflex function due to the longer epochs available.

1.4.1.1 Sequence Method. The sequence method is based on identifying the occurrence of sequences of consecutive beats, and is an often-used non-invasive technique for assessing baroreflex function. A sequence is defined as a progressive rise or fall in systolic blood pressure (SBP) with a subsequent lengthening or shortening of the R-R interval (RRI) for three or more consecutive beats [11,12,13,15,19,32]. If SBP

increases or decreases for three or more consecutive beats without eliciting the expected RRI response, then the SBP change is considered a stand-alone ramp. A valid sequence occurs when spontaneous SBP and the reflexive RRI move in the same direction for three or more beats.

When SBP increases, the baroreflex responds by lengthening the RRI, slowing down the heart and thus decreasing blood pressure as shown in Figure 1.8. Conversely, if SBP decreases, the baroreflex responds by decreasing the R-R interval (i.e., increasing heart rate) and raising blood pressure.

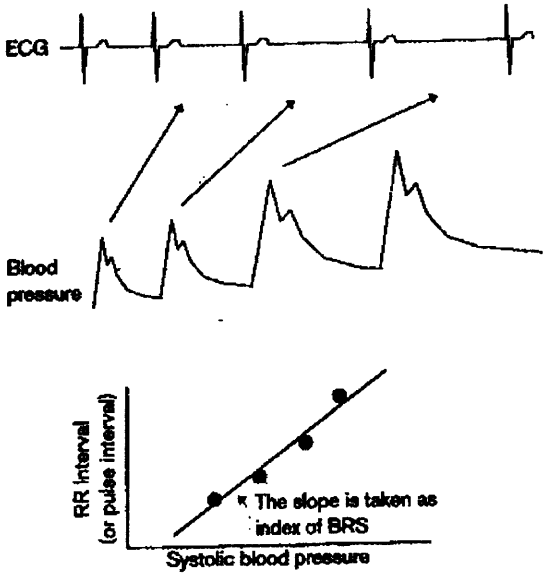


Figure 1.8 Schematic illustration of sequence method of baroreflex sensitivity. As blood pressure increases the interval between each R-to-R peak increases, thus decreasing blood pressure. The slope of the regression line is taken as BRSI [20].

The R-R interval response does not occur at the same time as the systolic blood pressure input. An inherent lag exists which can vary. With lag 0 sequences, the RRI response begins immediately following the change in SBP input. For lag 1 sequences, the RRI response begins one beat after the SBP input. For lag 2 sequences, the RRI

response begins two beats after the SBP input. Not all papers discuss lags; the majority of studies limit their analysis to lag 0 with little or no mention of other lags. The number of consecutive beats qualifying as a sequence also varies in the current literature. The discrepancy between researchers is whether three or four consecutive beats are necessary to qualify as a sequence [13 - 17].

The sequence method is based on computerized analysis of beat-to-beat measurements of SBP and RRI with lags of 0, 1 or 2 beats. When sequences are identified, the slope of the regression line between systolic blood pressure and the R-to-R interval is taken as an indication of the index of the arterial baroreceptors affecting heart rate or BRSI [11, 12, 14, 15, 16, 20]. When graphing the identified sequences, SBP is the independent variable, on the x-axis, in millimeters of mercury (mm Hg) with RRI the dependent variable measured in milliseconds (ms) on the y-axis. Figure 1.8 is a schematic illustration of the sequence method.

An additional measure that is easily calculated from the data collected during the sequence technique is the baroreflex effectiveness index or BEI. BEI is the ratio of the total number of sequences, which are identified as systolic blood pressure and R-to-R intervals changing in the same direction for three or more consecutive beats, to the total number of identified systolic blood pressure ramps [11, 13, 14, 20].

Systolic blood pressure ramps consist of three or more consecutive beats in which the SBP values rise or fall together. Not all ramps produce sequences. In order to calculate BEI it is necessary to maintain count of all SBP ramps regardless of whether or not they result in an RRI response. One study done by Di Rienzo et al. [13] showed that the behavior of BEI appears to provide complimentary information to BRSI. This thesis

will investigate what, if any, additional information can be obtained from BEI for this subject population. BEI quantifies the proportion of time the baroreflex is effective in driving heart rate based on the presence of SBP input ramps. BRSI is an indication of the gain or sensitivity of the baroreceptor and the heart rate reflex under spontaneous conditions.

1.5 Scope of Thesis

There has been only one other study identified that has addressed the evaluation of continuous cardiac baroreceptor measurement during HUT [28] and provided graphical representation of the baroreflex response. This study by Youde et al. is described in the Introduction of this chapter. The goal of this thesis is to analyze continuous cardiac baroreflex response during a head-up tilt study (HUT). The five groups evaluated are chronic fatigue syndrome with fibromyalgia, CFS-FM, chronic fatigue syndrome with postural orthostatic tachycardia syndrome, CFS-POTS, chronic fatigue syndrome, CFS, controls with postural orthostatic tachycardia syndrome, CON-POTS and controls, CON.

A comparative time analysis of weighted BRSI averages by group was done as well as a comparative time analysis of the weighted BRSI averages for lags 0, 1 and 2. Slope of BRSI over a two-minute moving window was evaluated by group over time. In addition, a comparative time analysis between groups for baroreflex effectiveness, BEI was done. Previous analysis of this type was not found in the literature. In addition, comparative analyses were done of the total number of sequences and ramps between groups, as well as between lags 0, 1 and 2 over time for the different patient populations.

Systolic blood pressure (the input) with the R-R interval the output response was evaluated at lags 0, 1 and 2 to determine baroreflex sensitivity index or BRSI. Di Rienzo

et al. [13] discussed the baroreflex effectiveness index indicating that BEI may provide complimentary information to BRSI. Thus, it became one of the goals of this thesis to identify whether the BEI provided additional information to distinguish between several patient groups.

The data consisted of 70 original study subjects, of which 25 were selected. The selection of 25 subjects was based on the availability of five chronic fatigue syndrome (CFS) subjects who also suffered from fibromyalgia (FM). As noted in Section 1.3 the LaManca study [3] some subjects were additionally afflicted with fibromyalgia and postural orthostatic tachycardia syndrome. Based on the diversity of disease states it was decided that the best exploratory analysis of BRSI was the selection of five groups made up of five subjects, all of whom as a group differed in their disease state. The five subject groups being evaluated in this thesis are five chronic fatigue syndrome (CFS), five chronic fatigue syndrome with postural orthostatic tachycardia syndrome (CFS-POTS), five chronic fatigue syndrome with fibromyalgia (CFS-FM), five controls with postural orthostatic tachycardia syndrome (CON-POTS), and five controls (CON).

CHAPTER 2

EXPERIMENTAL METHODOLOGY

2.1 Data Acquisition

The electrocardiogram (ECG) and the systolic blood pressure (SBP) recordings from the Finapres™ were the data utilized for this thesis. The ECG recordings were made using disposable silver/silver chloride (Ag/AgCl) electrodes using a modified lead II configuration [3]. The R-R interval (RRI) was derived from the ECG as the time between successive R spikes for each beat (see Figure 1.7). The SBP readings used represent the peak of the blood pressure waveform (see Figure 1.3). These values were obtained via SPlus programs written by Michael Bergen of the East Orange Veterans Administration Medical Center.

The control subjects in the original study were matched as closely as possible to the CFS subjects such that they were healthy but physically inactive [3]. Ten of the CFS subjects also had postural orthostatic tachycardia syndrome (POTS), and five CFS patients had fibromyalgia (FM). Six of the thirty-one healthy controls had POTS. The goals of the original study were to evaluate the diagnostic value of head-up tilt (HUT) in chronic fatigue syndrome as well as using HUT to understand cardiovascular autonomic function in CFS [3]. In order to assess cardiovascular autonomic function, thoracic impedance cardiography was used to derive stroke volume, myocardial contractility and total peripheral resistance [3]. This thesis deals only with the electrocardiogram (ECG) and blood pressure measures. Blood pressure was continuously measured throughout the course of the study using a Finapres™, Ohmeda Model 2300. The Finapres™, as

described in Section 1.2.1, uses an inflatable finger cuff with a built-in photoelectric plethysmograph [4].

The finger cuff was placed on the index finger of the subject's left hand and was kept at heart level by means of an adjustable arm support [3]. A Dinamap™, Model 1846 SX blood pressure monitor, recorded blood pressure during each recording period. The Dinamap™ was used as a gold standard for this study. Thus when the reading was available, it was compared to the Finapres™ reading. Based on the comparison adjustments were made to either the finger cuff of the Finapres™ or the arm height until the values were similar [3].

The Finapres™ calibrates itself automatically once every 70 beats, or approximately once every minute [4]. It is accepted practice to disable the calibration circuit when continuous blood pressure readings are desired. During the course of the original study, the automatic calibration was not consistently disabled leaving gaps in the systolic blood pressure readings. This posed a problem in analysis and algorithm development, which is discussed in Section 2.2.

2.2 Methods of Analysis

The first step in processing the data was to organize the data to be analyzed into a spreadsheet file for each subject using Excel. The spreadsheet file contains four columns; the systolic blood pressure (SBP) values in mm Hg, the R-R intervals in milliseconds, the SBP index or sample value and the RRI index. These latter two indexes indicate the time expressed in samples for the SBP value and the R-R interval. Figure 2.1 illustrates the

spreadsheet form. There were two files for each subject, an ECG and a blood pressure file. The sampling rate for all data was 200 samples per second.

The original ECG file contained two columns of data, the first the sample time and the second the R-R interval both written in seconds. The sample times were multiplied by the sampling rate, 200 samples per second, to convert each point to a sample index. The R-R interval values were multiplied by 1000 to express RRI in milliseconds, which is standard. The recalculated columns were copied to a new spreadsheet file with the RRI sample index placed in the fourth column and the RRI values in the second column (see Figure 2.1).

SBP (mm Hg)	RRI (ms)	SBP Index	RRI Index
123	460	151	92
125	770	305	246
123	820	468	410
118	860	642	582
121	815	805	745
121	805	966	906
121	860	1138	1078
121	820	1302	1242

Figure 2.1 Example of four-column spreadsheet configuration.

The blood pressure files for each subject contained numerous columns of information including diastolic and systolic pressure values as well as sample index arrays for each. As noted, this thesis is only concerned with the systolic blood pressure readings in keeping with prior studies of spontaneous BRS (measured from the BP-RRI relationship) which use SBP. The SBP sample index was copied directly to the third column of the new spreadsheet. The SBP value array was also directly copied, however

prior to being placed in the first column of the new spreadsheet, the round function in Excel was used to eliminate the fractional component. Once the SBP values were rounded, these values were then copied to the first column of the spreadsheet using copy, paste special, values only and the original array was deleted. This procedure was followed for each subject. As mentioned earlier, the Finapres™ calibration circuit was not disabled during the bulk of the continuous data acquisition, thus gaps in the SBP readings existed. Editing errors also existed in both the RRI and SBP files. Two factors contributed to editing errors, misinterpretation by the algorithm during the peak detection process and human error during manual verification of the algorithm output.

Once the Excel files were prepared for each subject, it was necessary to identify the location and number of the editing errors and the gaps for each file. Difference arrays were calculated by subtracting the first value of the sample index array from the second value of the same array, the second value subtracted from the third and so on. This was done for both the RRI and SBP sample index arrays, with calculations done in Excel. Because array sizes were large, it made sense to graph the difference values to identify gaps and editing errors; graphing also assisted in algorithm development. When the calculated difference arrays were graphed, large upward spikes identified gaps in the SBP due to the Finapres™ calibration circuit. Downward spikes identified editing errors where two SBP peaks or two R spikes were identified too close together to be real separate values. The RRI data only contained editing errors; there were no RRI gaps identified because the Finapres™ calibration pauses affected only the SBP data. Figure 2.2 is an illustration of SBP gaps and editing errors as graphed in Excel.

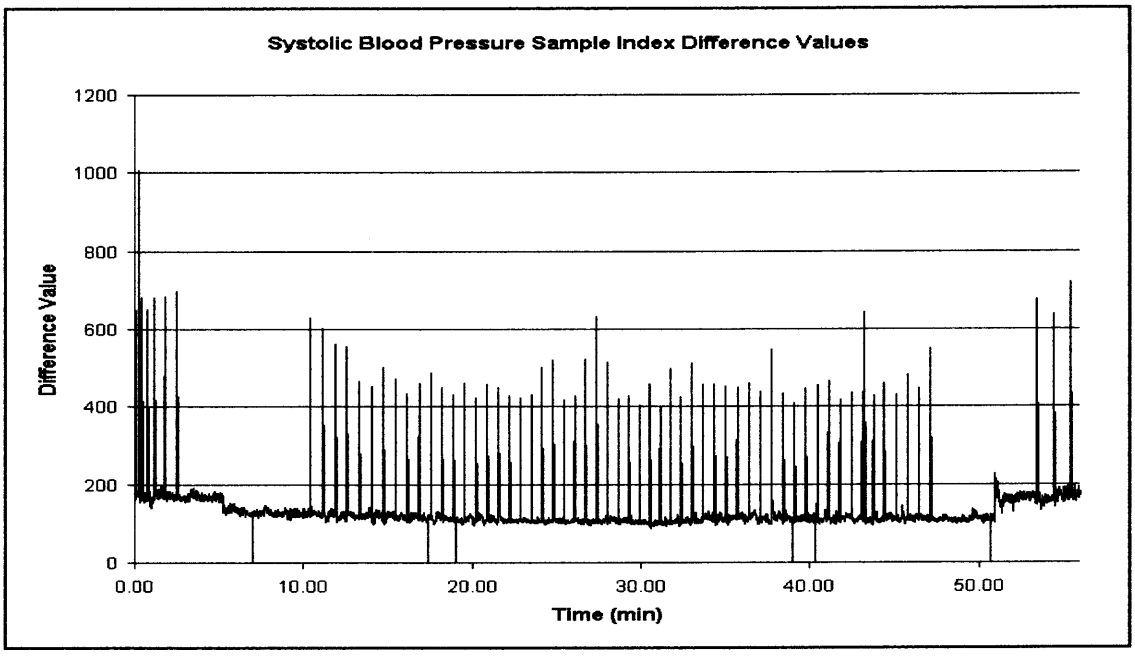


Figure 2.2 Difference values of SBP index array. Spikes upward are gaps, downward are editing errors.

Gaps in the systolic blood pressure and editing errors in both the SBP and RRI data were manually corrected in one file that served as a template for algorithm development. For subsequent files, computer algorithms were used to correct the gaps and extra beats as described next.

Two programs were developed to deal with the problems illustrated in Figure 2.2. The first program checks for and removes editing errors, i.e., the downward spikes. This algorithm, named the eliminator, removes editing errors based on user input defining acceptable thresholds for the time between detection of R spikes and SBP peak values. The second program interpolates the data, or fills in the gaps thus removing the upward spikes in Figure 2.2. An interpolator program was developed to fill in the gaps in the SBP file to maintain the integrity of the time line.

The last step, which did not occur until all programs were operating correctly, was the development of a program to group the data further analysis. The data is grouped based on user input, which must be an integer value in minutes. A flow diagram of the overall process is shown in Figure 2.3. Descriptions of each program are provided in Section 2.2.1. In addition, Appendix B illustrates the front panel and the high-level block diagrams of the key programs with a brief description of the main function for each.

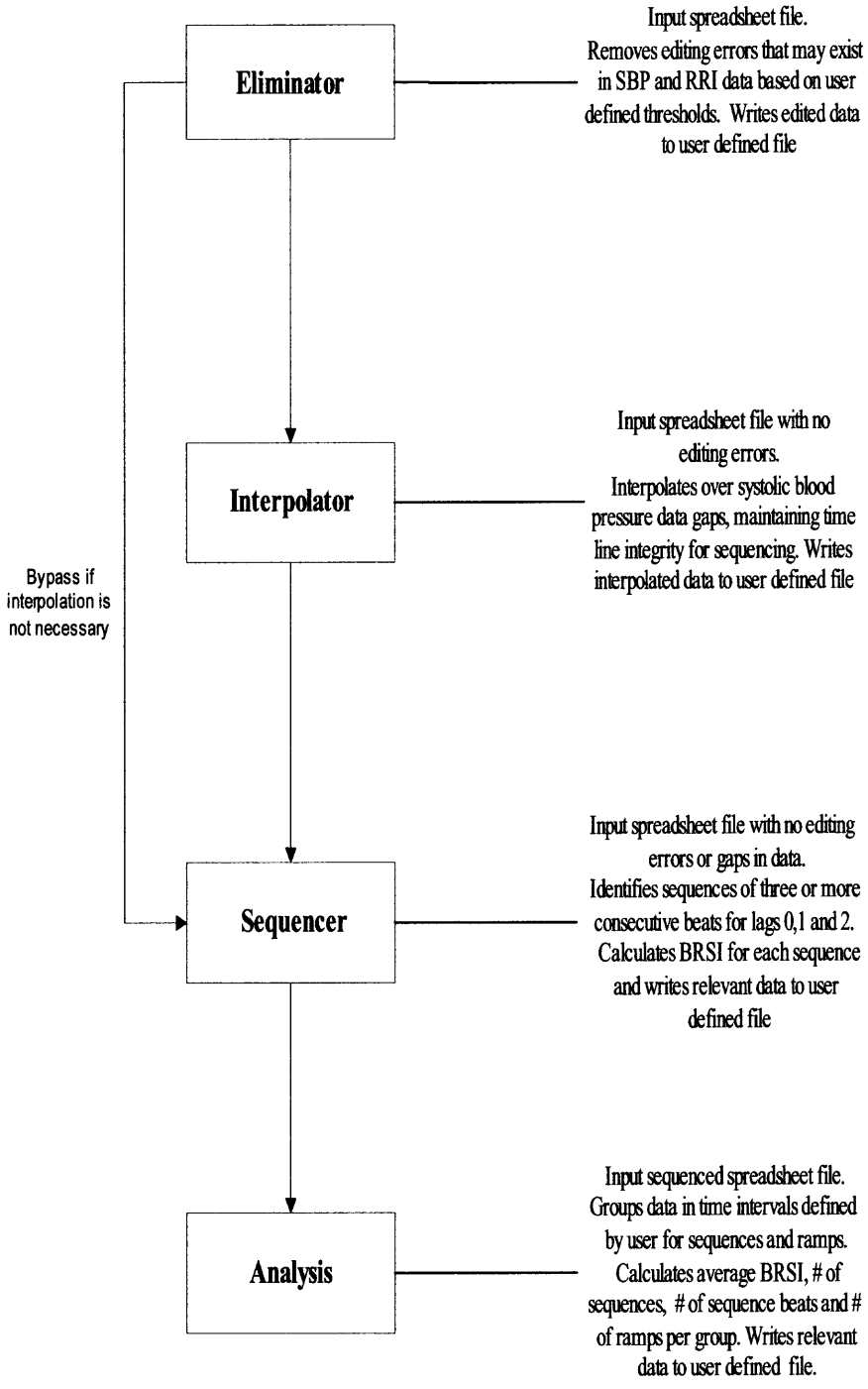


Figure 2.3 Top level block diagram indicating program flow for sequencer system. All programming was done in LabVIEW version 6.1.

2.2.1 Block Descriptions

The first algorithm shown in Figure 2.3 is the eliminator program. It was written to remove editing errors. Editing errors would appear due to computer error based on the program written to identify peaks in both the ECG and SBP recordings and human error when the interval files were manually checked. The program reads in the four-column spreadsheet defined in Section 2.2 and calculates two difference arrays. Programmatic calculation of a difference array is shown in Figure 2.4. Array 1 was made by shifting the original array up by one and removing the zero index value. Array 2 was made by removing the last value of the original array. Array 2 is then subtracted from array 1 giving the difference values of the original array.

Original Array	Array 1	Array 2	Difference Array
121	119	121	-2
119	117	119	-2
117	122	117	4
122	126	122	4
126			

Figure 2.4 Sample of difference array calculation.

This process is done for both the SBP and the RRI sample index arrays. The calculated difference values are compared to threshold values that are defined by the user. There are two threshold inputs, one for the systolic blood pressure index and the other for RRI index. If the calculated difference is greater than the defined threshold, the original value is undisturbed and is passed through the program intact. If the calculated difference is less than the defined threshold value the algorithm removes the appropriate value in both the sample index and its corresponding RRI or SBP value in the array.

The program discriminates between SBP and RRI editing errors, making only the corrections that are required. The thresholds must be defined by the user and can be any integer value. The eliminator program contains four sub-vi's facilitating operation. The program rewrites the corrected data to a file defined by the user. If no editing errors are identified the original arrays are undisturbed; however, the user is still prompted at the end of the program to define a save to file destination. After saving to a file, program operation can be verified by calculating difference arrays of the eliminator output and then graphing them in Excel. Figure 2.5 illustrates the data originally shown in Figure 2.2 after the file was run through the eliminator program. The downward spikes representing editing errors that were present in Figure 2.2 are removed and only upward spikes or SBP gaps remain.

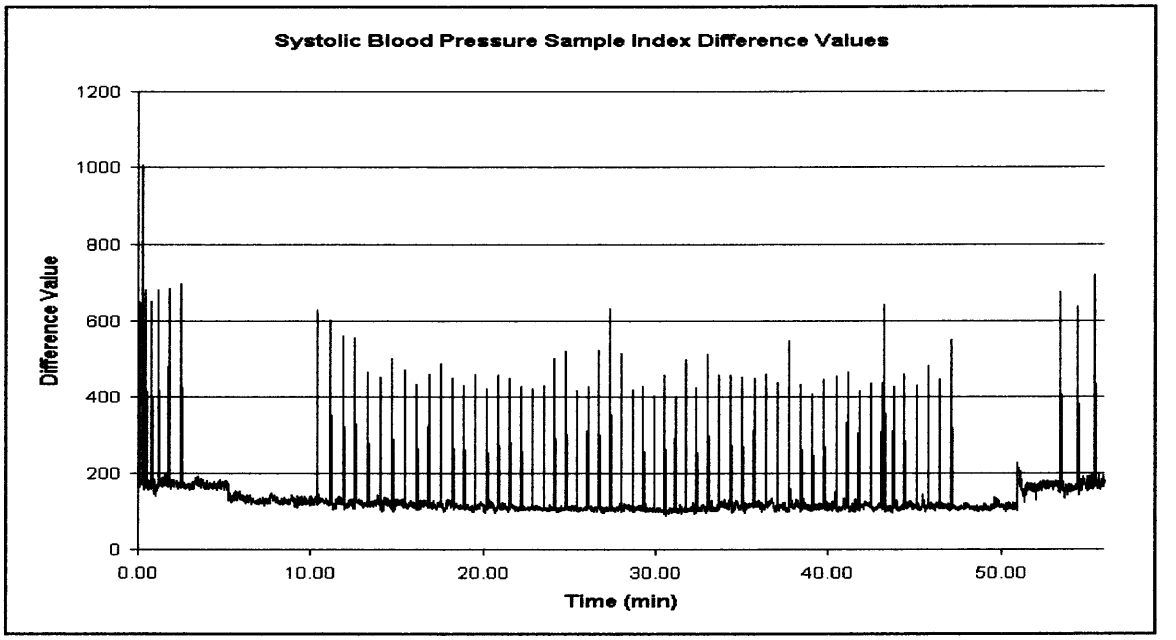


Figure 2.5 SBP difference values of sample index with editing errors removed.

2.2.1.1 Interpolator. The interpolator was written to address the specific problem of gaps in the systolic blood pressure readings, which as mentioned earlier were due to the Finapres™ calibration circuit. The interpolator will not compensate for gaps in RRI data. The main purpose of the interpolator is to reconstruct the systolic blood pressure time line by expanding the SBP sample index array so that the data can be sequenced. The interpolated SBP pressure values (mm Hg), are not considered when determining sequences (discussed in Section 2.2.1.2).

When the interpolator runs, it prompts the user for a file to read. The file must be free of editing errors and in the four-column format described in Section 2.2, and shown in Figure 2.1. The interpolator cannot process data that contains editing errors. Once a valid file is selected, the four-column spreadsheet is separated into four arrays. The SBP and RRI index arrays are used by the sub-routine, shown in Figure 2.6, to identify gaps.

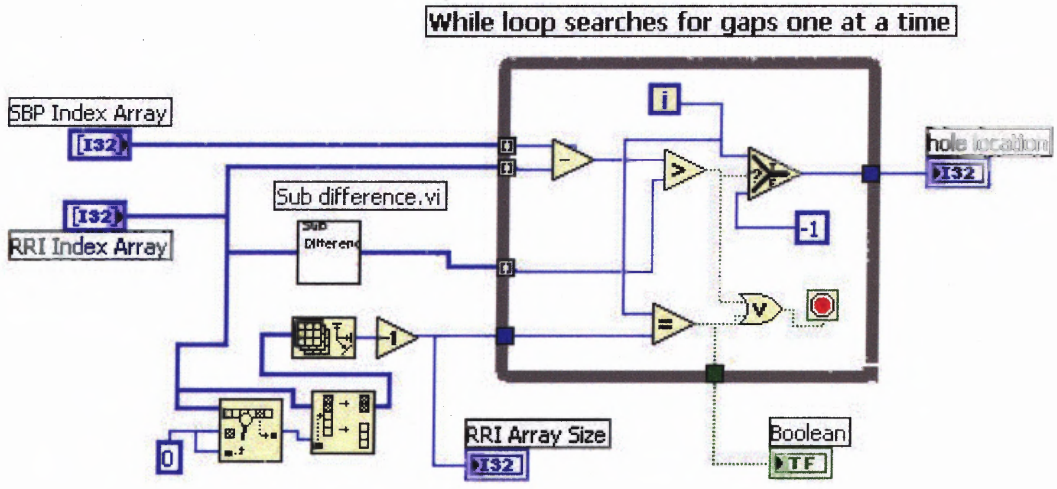


Figure 2.6 Algorithm detecting gap locations. The while loop searches for gaps in the SBP array, returning the index value of the gap location. This location is used by another subroutine to fill in the gap. This process continues until all gaps are filled.

The subroutine in Figure 2.6 identifies gaps by calculating a difference array from the RRI sample index array. The difference array calculation was described in Section 2.2.1, and shown in Figure 2.4. A difference array is calculated from the RRI index value array. The difference array is compared to the SBP index array minus the RRI index array on a point-by-point basis. If the subtracted value (SBP array minus the RRI array) is greater than the difference array value then a hole exists. If the difference is not greater, the comparison continues.

If no gaps are identified the file is untouched; if a gap is identified the while loop stops and the subroutine points to the gap location by outputting an index value. This index value is used by another sub-routine to fill in the first value of the gap. If the gap extends beyond one value, as it generally does, the process continues filling one value at a time until each gap is completely filled. After the program has run, it prompts the user to save the interpolated file. The file name and destination is user definable. Interpolation can be verified by graphing the SBP difference array of an interpolated file, which is shown in Figure 2.7 below (see Figure 2.5).

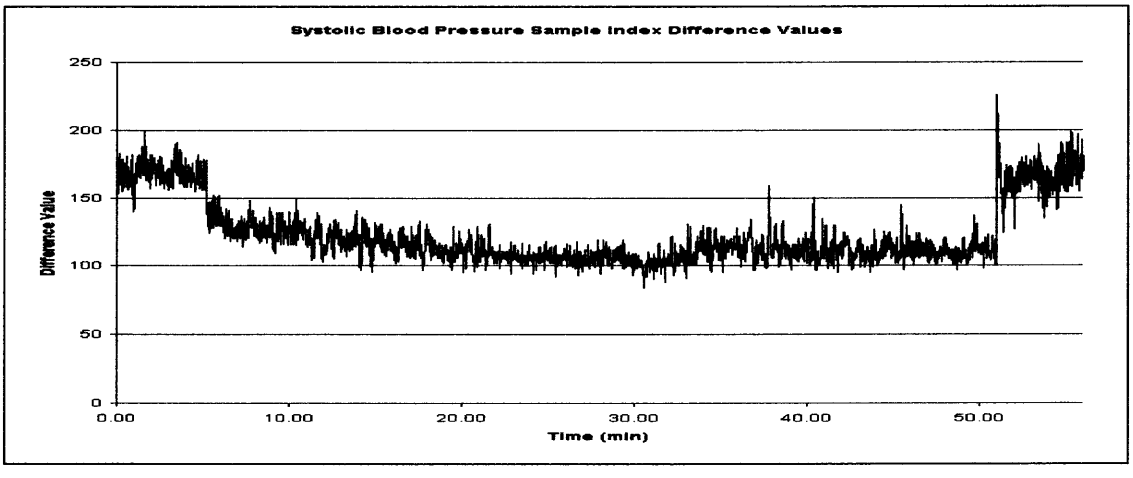


Figure 2.7 SBP difference values of sample index. Gaps and editing errors removed.

The interpolator saves the file in a five-column format. The first four columns are in the original format as shown in Figure 2.1; a fifth column contains the index values of the last interpolated data point. The location of the last interpolated value is critical to development of the sequencer algorithm.

The main problem during development of the interpolator was defining the optimal method to identify gaps. Identifying gap locations all at once and storing these locations in an array for use by supporting subroutines caused a cascading problem. After the first gap was filled, subsequent gap locations were invalid, all being displaced by the number of times the initial gap needed to be filled. Although several solutions were attempted, it was found that filling the gaps one value at a time was the optimal approach. The interpolator consists of the main program and seven supporting sub-routines.

2.2.1.2 Sequencer. There are three sequencers, one for each lag. Each sequencer contains numerous supporting subroutines to form the main sequencer program. The sequencer begins by reading in a five column interpolated file. The sequencer can read and sequence data that has not been interpolated as long as there are no gaps or editing errors and the column format as described in Section 2.2 is followed.

The SBP index array and the fifth column of the interpolated file, which is the index value of the last interpolated data point, are used to identify valid segments. Segments are systolic blood pressure values that increase or decrease for one or more intervals, or two or more beats. Segments are identified by scanning the systolic blood pressure data looking for consecutive increasing or decreasing values of two or more beats. When segments are located the begin and end index values corresponding to these

increasing or decreasing sections of the data are stored in two arrays. After all SBP data has been scanned and segments identified the fifth column of the interpolated file is compared to the begin index array of the segments. If there is a match this means that a segment has been identified as beginning on an interpolated SBP value. Since the interpolated data is not valid biological information, it cannot be considered when determining sequences.

When a match is found between a segment begin index value and an interpolated index value the begin index value for the segment is adjusted. For example if segments are identified as beginning on index values 10, 15 and 20 and index value 10 is identified as an interpolated data point, the value 10 is removed from the segment begin index array and the next value, 15 is copied in its place. The new segment begin index array has become 15, 15, 20. This procedure maintains consistency in array sizes for begin and end index arrays and eliminates the possibility of a segment, and as a result a sequence, from beginning on an interpolated data value. If the fifth column does not exist, such as in a non-interpolated file there are no comparisons to be made, and the program runs normally. Because segment identification is based on increasing or decreasing SBP values, and interpolated SBP values remain constant, it is impossible for a segment to end on an interpolated value, therefore only the begin index values are checked.

The begin and end segment index arrays are used by another subroutine that identifies ramps from segments. The distinction between a ramp and a segment is the additional criterion that a ramp must last for a minimum number of beats. The minimum number of beats to define a ramp from a segment is user definable. The input was made variable due to the different preferences that exist between researchers in the field of

baroreflex sensitivity. This thesis has used a minimum of three beats to define a segment. Once all ramps are identified from the available segments they are stored in arrays of beginning and ending index values. The ramps beginning and ending points are processed by another subroutine to identify sequences from ramps.

The process of identifying sequences from ramps requires comparing the SBP and RRI value arrays in sections demarcated by the begin and end points of each ramp. In order to accomplish this two difference arrays (as previously noted) are calculated for both the SBP (or x array) and the RRI (or y) value arrays. The two difference arrays are then multiplied together forming a new array (x*y). This new array provides directional information between the SBP and RRI arrays. A zero would mean one or both (x and y) arrays were zero indicating no concomitant change in direction at these points. A negative number would indicate a change in direction.

The begin point of each ramp is used to index the (x*y) array, for a length defined by the difference between the end and begin index points of each ramp. Each section of the (x*y) array is checked to see if the values are moving in the same direction, if they are and the directional change lasts for three or more beats it is considered a valid sequence. If the arrays do not increment in the same direction or if they do not increment for at least three beats then that SBP ramp has not produced a valid sequence and the program moves on to the next ramp. This process is done for lags 0, 1 and 2.

Identified sequences are stored as index values of beginning and ending points for each sequence, thus the number of beats per sequence is easily obtained. The begin and end index arrays for each sequence are used by a regression sub-routine which takes the SBP and RRI value arrays in sections as dictated by the begin and end indices of each

sequence and calculates the best linear fit for these values. The slope of this regression line is taken as the baroreflex sensitivity index.

Before writing the data to a file, the sequencer calculates the time in minutes for each sequence. This is done by equating both begin and end index arrays for each sequence to the systolic blood pressure index value, which represents the sequence in terms of samples. Dividing each sample index by the sample rate of 200 samples per second gives the sequence begin and end time in seconds, dividing again by 60 gives the time in minutes. This conversion to begin and end times is also done for all ramps regardless of whether or not they qualify as a sequence. When the sequencer has performed all of its functions, it prompts the user to save to a file.

The sequencer writes header information as well as data for lags 0, 1 and 2. Header information includes subject identification, time and date of analysis, total number of ramps, total number of segments and the total number of sequences for each lag. In addition, four columns of data are written to file for each lag. The format is illustrated in Figure 2.8. Two additional columns identifying the begin time in minutes of each ramp and the number of beats in each ramp are written to file along with the sequenced data and appear in the spreadsheet after lag 2 data. The sequencer consists of the main program and thirty supporting sub routines.

Subject t146	6/12/2003	10:57 AM	
Ramps = 1208	Segments = 1929	Beats = 3	BEI Lag 0 = 0.402
Lag 0	204 Sequences		
Begin Sample Time	Begin Sample Index	BRSI	Sequence Size
0.18	2155	4.863	3

Figure 2.8 Example of sequenced information written to file. Output for lags 1 and 2 is identical.

Numerous problems arose in the development of the sequencer. Identification of segments, one of the more critical steps in the process was one of the first serious problems encountered. If segments were improperly identified the remaining processes would produce incorrect results. Tests were performed on each program developed prior to implementation with the intent of trying to force the program to fail. As each programming step was completed, the full program was assembled and the assembled portions tested again. When the program was implemented with a complete data set, the outputs were manually spot-checked for accuracy, and any identified problems corrected. Early indicators that problems existed were the presence of negative slopes for BRSI, extremely large BRSI and duplicate BRSI values. Problems were approached in steps until all were resolved.

One problem became evident when the baroreflex effectiveness index (BEI) was calculated. BEI is a ratio of the total number of sequences divided by the total number of ramps. This percentage is an indicator of the effectiveness of the baroreflex control of the heart rate [13]. By definition, BEI should not be larger than one. When calculation of BEI began returning values greater than one, a deeper investigation into the sequencer program was done. The initial problem identified was two fold; first, the sequencer was not identifying all available sequences and in some instances when it did identify sequences they were undersized by one beat. The source of this problem was due to the way the sequencer was asked to make its decisions.

With segments and ramps properly identified, it is necessary to compare the SBP and RRI value arrays to determine when sequences result from ramps. This process was described previously but briefly, a difference array is calculated for both the SBP and

RRI arrays. The resulting difference arrays are then multiplied together forming a new (x*y) array. When a difference array is calculated, the resulting array has one less value than the array from which it was calculated, (see Figure 2.4). As a result, when sections of the (x*y) difference array are used to check for sequences the last value of the array actually reflects the comparison one point beyond the ramp being evaluated. What this means is if an identified section of the (x*y) array ends in a negative or zero value, then a value of one should be added to the number of beats that the program calculates prior to determining if the sequence is valid. Changes to the subroutine that calculates sequences from ramps to account for the above problem were made. This change increased the number of identified sequences dramatically because two-beat sequences that were previously discounted became valid sequences. Extensive manual checks of the new output were done to locate problems and to insure the repair was correctly implemented.

The second problem led to the discovery that the sequences between each lag were not mutually exclusive. There is one group of ramps available for all lags; these ramps were being used by lags 0, 1 and 2, in parallel to produce valid sequences. Based on previous literature, it was recognized that lags had to be mutually exclusive in order to provide valid results. Subroutines had already been developed and implemented to remove redundancies based on common begin points for sequences between lags. It was believed that the mutually exclusive requirements were being met. Although this appeared to work, it did not go far enough and another solution needed to be developed.

Up to this point each sequencer, one per lag, were running in parallel. Each sequencer identified the same segments and ramps from the input data and each had

complete access to all available ramps. The solution involved running the sequencers in series with the lead sequencer identifying segments and ramps.

The idea was to allow lag 0 to have access to all ramps, lag 1 to have access only to the ramps left over from lag 0 and lag 2 to have access only to the ramps remaining after lag 1. A subroutine was developed to keep track of ramps and sequences as they were identified. Any ramp that produces a valid sequence is marked along with any sequence beyond the first that is produced by a single ramp. If two or more sequences are produced from one ramp only the first sequence is considered valid with subsequent sequences marked for elimination. After all sequences are identified, the marked ramps and sequences are eliminated. The total number of sequences recorded as occurring within a particular lag is now mutually exclusive, consisting of one sequence per ramp. The remaining ramps begin and end index values from lag 0 are then fed into the next sequencer, in this case lag 1. The lag 1 sequencer no longer identifies segments, it only judges if the available ramps produce a lag 1 sequence. It goes through the same elimination process as the lag 0 sequencer feeding its remaining ramps to the lag 2 sequencer. Lag 2 sequencer operation is identical to the lag 1 sequencer.

After the subroutine was written and tested, it was placed in each sequencer, one for each lag. The new subroutine also made obsolete the programming previously done which tested for duplicate sequences based on the sequence start point. This now obsolete programming was removed and each sequencer was reformatted and rewired to accommodate all changes.

2.2.1.3 Analysis Programs. The analysis program reads in a sequenced spreadsheet and groups the data based on a user defined time interval in minutes, which must be an

integer value. The program initially requests the user to identify a file to read. It then reads in the file, processes the data and writes its results to a file defined by the user. An example of the data written to file is shown in Figure 2.9.

Subject t380	7/17/2003	7:45 PM	Grouping (min) 1	
Lag 0				
Begin Time	End Time	Avg. BRSI	Seq. in Group	Seq. beats in Group
0	1	12.486	2	6
1	2	14.096	5	15
2	3	14.387	3	9
3	4	13.396	10	30
4	5	9.583	3	9
5	6	6.807	7	21

Total Ramps			
Begin Time	End Time	Ramps in Group	Beats in Group
0	1	9	27
1	2	14	42
2	3	13	39
3	4	16	48
4	5	14	43
5	6	24	77

Figure 2.9 Example of the analysis data written to file. Above is the data written to file for each lag. The bottom of the figure illustrates the data on ramps, which is written to the file appearing after the lag 2 data.

Figure 2.9 illustrates the output of the analysis program based on groupings of data in one-minute intervals. The top section of Figure 2.9 indicates the header information written to file for each subject. The begin and end times, the average BRSI value, the number of sequences, and the number of sequence beats are all displayed by lag for each identified interval. The bottom of Figure 2.9 is the ramp information written to the file. Ramps begin and end times, the total number of ramps and the total number of ramp beats for each group are displayed. Ramp information is written to the same file in columns following lag 2 data.

The program reads in a sequenced file spreadsheet and separates from it three arrays for each lag, the sequences begin time, the BRSI value and the number of beats per sequence. Ramp information is also separated from the sequenced file for analysis. The three arrays for each lag are fed into a grouping analysis subroutine, one for each lag, that first determines the begin and end index values of the specified time interval based on the sequence begin time array. Index locations identifying begin and end points for each interval are used by supporting subroutines to index the BRSI and segment beat arrays to calculate an average BRSI value for each time interval along with the total number of beats per interval. Average BRSI is calculated for each interval by indexing the BRSI array at the begin index value of the interval, for a length equal to one plus the difference between the begin and end index locations. The BRSI values are then summed and divided by the length (end index minus begin index). The total beats per interval are the summation of the array values in the identified interval. Ramp information is processed the same way except there is no average calculation done, only summation of number of ramps and ramp beats per interval based on the ramp begin time.

After each sequenced file has been run through the analysis program Excel was used to perform analysis of the data. Initially a total group weighted average was calculated for each interval. Group weighted average was calculated by multiplying the BRSI value for each interval by the number of beats for each interval, for each lag. The products from each lag were added together, and then divided by the total number of beats for that interval.

A new Excel workbook was compiled containing 20 spreadsheets, four spreadsheets of data for each disease state combining the results of the five subjects in

each disease group. The four spreadsheets compiled for each group were the total group average, group average by lag, total number of ramps per group and the number of sequences per group for each lag. An example of the four-spreadsheet groupings for one subject is shown in Figure 2.10. Sequences do not necessarily occur for each lag. Presence of a lag 2 sequence does not mean a lag 1 sequence should also occur. This is why there are blanks in the lag data in the bottom of Figure 2.10.

	Group BRSI Weighted Averages 1-min intervals by subject				
End Time					
(min)	t125	t182	t211	t214	t235
1	36.445	12.944	18.063	9.481	7.307
2	42.998	10.823	12.073	9.161	8.135
3	23.220	12.300	13.923	6.263	6.624

	CFS - Group Average BRSI by Lag 1-min intervals by subject					
			t125			
	Lag 0		Lag 1		Lag 2	
End Time	Avg. BRSI	seq beats	Avg. BRSI	seq beats	Avg. BRSI	seq beats
1	37.685	13			31.071	3
2	42.998	14				
3	23.22	12				

	Group BRSI Ramps 1-min intervals by subject				
End Time					
(min)	t125	t182	t211	t214	t235
1	8	8	8	16	22
2	8	14	13	18	21
3	6	14	13	20	20

	Group Sequences 1-min intervals											
End Time		t125				t182				t211		
(min)	Lag 0	Lag 1	Lag 2	TL	Lag 0	Lag 1	Lag 2	TL	Lag 0	Lag 1	Lag 2	TL
1	4		1	5	6			6	4			4
2	4			4	9		1	10	10	2		12
3	4			4	7		1	8	6	1	1	8

Figure 2.10 Sample of compiled spreadsheet files done for each group, four spreadsheet files for each group. The groupings by lag are repeated for all five subjects within the group.

CHAPTER 3

RESULTS

There has been only one study identified to date that evaluates BRSI over time during head-up tilt. This study done by Youde et al. [28] evaluated continuous baroreceptor measurement in healthy elderly individuals. Each subject, on two separate occasions, underwent three head-up tilts to 70^0 that lasted for 10 minutes each. Further, a standardized sequence length of three beats was used and only lag 0 BRSI was evaluated. For this thesis, head-up tilt was also 70^0, however it lasted for 45 minutes unless presyncopal or syncopal symptoms were exhibited by the subject. All data was gathered in one visit and lags 0, 1 and 2 are evaluated. In addition, the disease state CFS was compared to matched controls to assess the utility of looking at the BRSI changes over time across the disease groups.

This section presents results of the observational analysis of data for all groups for the specified intervals. Here, the term group refers to the subject groupings chronic fatigue syndrome, CFS, chronic fatigue syndrome with postural orthostatic tachycardia syndrome, CFS-POTS, chronic fatigue syndrome with fibromyalgia, CFS-FM, control with postural orthostatic tachycardia syndrome, CON-POTS and control, CON. The output from the analysis program was analyzed in one-minute epochs to smooth the BRSI results over time. Theoretically, grouping can be done in any time interval as defined by the user prior to running the analysis program. Use of the term interval here refers to the one-minute intervals selected for evaluation in this thesis.

When necessary for clarity, supplementary graphs are shown in Appendix C. These supplementary graphs break down the data into three sections beginning, middle and end. The beginning graph is of the first 15 minutes, which includes five minutes of baseline and the first 10 minutes of tilt. The middle graph represents 15 to 41, which is exclusively tilt data. The end graph is the end of the test, minutes 41 to 46, which includes the last five minutes of tilt and the entire recovery period. Sectioning the data in this manner provides sharper contrast of the transitions between baseline and tilt, and tilt and recovery as well as illustrating the variability between groups during the entire test. Appendix A contains a table of each subject identifying individual time intervals for baseline, tilt and recovery periods.

The head-up tilt test began by collecting baseline data with the subject secured on the tilt table in a supine position. At approximately five minutes into the test, the subject was manually tilted in less than four seconds to a 70^0 angle. The tilt lasted for 45 minutes, unless the subject developed syncope or presyncopal symptoms [3]. When the subject was returned to supine a recovery period of roughly six minutes occurred. A successful test contained 56 minutes of data for each subject.

The final analysis program compiled subject data in one-minute intervals and calculated for each interval an average BRSI, the total number of sequences, and the total number of sequence beats. This was done for lags 0, 1 and 2. The total number of ramps for each one-minute interval was also compiled with all results written to an Excel spreadsheet.

3.1 Analysis of BRSI Over Time

Once all data was processed using the analysis program a weighted BRSI average was calculated for each group. The weighted average was calculated by multiplying the average BRSI value for an interval by the number of sequence beats. This was done for each lag in the interval. These products were summed and divided by the sum total of the sequence beats within that interval for all lags. This provided one BRSI weighted average array for each group in order to compare groups.

Initially, analysis was performed by averaging the weighted averages for each subject within each group. The differences between the two analysis techniques were subtle but averaging a weighted average may be a misrepresentation of BRSI amplitudes and as a result slopes. Graphs of the group BRSI and the associated slopes using this calculation technique are shown in Appendix C as a comparison to illustrate the differences between the two methods. It is believed that calculation of a true weighted BRSI average provides more accurate results thus better comparisons between groups.

Figure 3.1 illustrates a 56-minute graph of the results comparing the weighted average of BRSI across groups. The graph is marked indicating the three periods of HUT, baseline, head-up tilt and recovery. A graphical analysis of the BRSI values overtime for the entire HUT shows a similar trend for each group. Each group exhibits higher average BRSI values, when supine then during head-up tilt. These results are consistent with previous research [28].

When subjects are tilted, there is a significant drop in BRSI values indicating decreased baroreflex sensitivity during tilt. Although some variability in BRSI is present during HUT, the lower sensitivity remains consistent throughout the 45-minute tilt test.

Conversely, a significant increase in BRSI values is evident when the subject is returned to supine indicating an almost immediate improvement in the sensitivity of the baroreflex. Figure 3.1 is sectioned to indicate the three phases of the test, baseline, HUT and recovery.

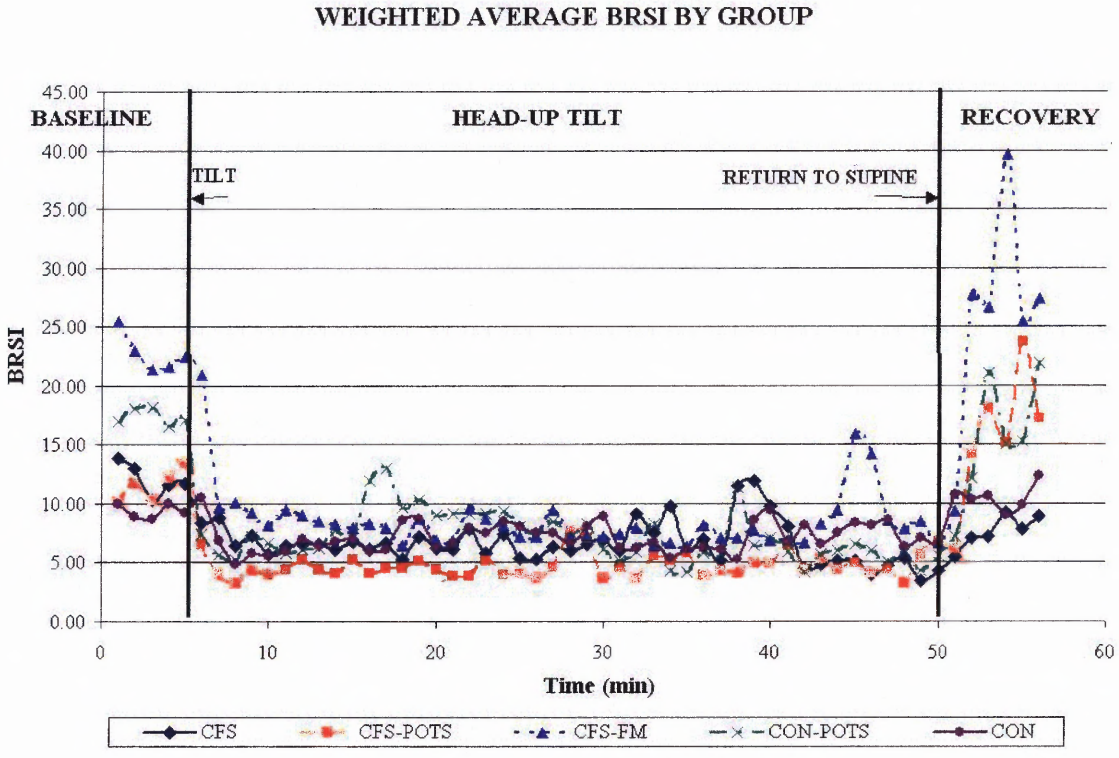


Figure 3.1 Graph of weighted average for BRSI during head-up tilt for all subject groups. The graph is marked to indicate the three stages of HUT.

During baseline the group with the highest BRSI values is chronic fatigue syndrome with fibromyalgia, CFS-FM, the lowest values belong to the control group, CON. The chronic fatigue syndrome group, CFS, is between these two extremes. The controls with postural orthostatic tachycardia syndrome, CON-POTS, are above the CFS group and chronic fatigue syndrome with postural orthostatic tachycardia syndrome,

CFS-POTS, varies about CFS. During tilt the variability of all groups follow similar patterns with the exception of oscillations present in CON-POTS, CFS and CFS-FM groups. These oscillations are all due to subjects within the respective group whose test ended prematurely due to presyncopal or syncopal symptoms. The premature recovery experienced by these subjects results in the spikes.

Although BRSI varies during tilt, it does not begin to recover supine levels for any of the subject groups until recovery. During the six-minute recovery period, CFS-FM, CFS-POTS and CON-POTS all rise abruptly with amplitudes above their baseline values. CON and CFS do not exhibit the same behavior. CON has slightly higher values in recovery than they did at baseline; CFS is below baseline values. CFS and CON groups follow similar patterns through the entire HUT. Sectional graphs that break down the 56-minute HUT into three sections, beginning, middle and end provides a sharper view of the contrast between subject groups, and are shown in Appendix C.

A previous study using a 60^0 head-up tilt reported that a significantly larger number of baroreflex sequences were present during tilt versus supine, with a decreased baroreflex sensitivity [15]. In order to see if there was general agreement between the combined data of this study and the sequence pattern described in the previous study sequences were totaled across all groups for specific intervals. The time intervals totaled across all groups were the five-minute baseline data, the first five minutes of tilt, the last five minutes of tilt, and the first five minutes of the six-minute recovery period. The total number of ramps available for each time interval is also included. The totals are shown in Table 3.1. This indicates a general agreement with the results reported in the 60^0 tilt study [15].

Table 3.1 Sequences for equal periods between supine and HUT.

Time Interval	TL. Supine Sequences	TL. Tilt Sequences	Available Ramps
1 - 5 min	1334	N/A	1930
6 - 10min	N/A	1390	2443
46 – 50 min	N/A	946	1673
51 – 55 min	719	N/A	1202

Referencing Table 3.1, an increase of 56 sequences, an approximate 4% increase, occurred during the first five minutes of tilt as compared to the first five minutes of baseline (supine). A decrease of 227 sequences occurred during the first five minutes of recovery as compared to the last five minutes of tilt, an approximate 24% drop. If the comparison during recovery included the full six-minute recovery period, the number of sequences during recovery is still lower (819) than the total number of sequences during the last five minutes of tilt.

Di Rienzo's BEI study [13] also reported a reduction in the number of sequences at night as compared to day recordings on 24-hour ambulatory subjects. When the body is in a supine position, there is less effort due in part to reduced vascular resistance, to provide consistent blood flow to critical organs. During tilt, this "effort" increases as is evidenced by the increased number of ramps and sequences during tilt, yet the sensitivity of the baroreflex decreases. The rationale for this was not identified in the literature. This may be due to where a person is on the baroreflex curve under these different postures.

3.2 Analysis of BRSI Slopes Over Time

Slopes of the weighted averages for BRSI were calculated over a moving window of two-minute intervals. A two-minute window was selected because a larger window seemed to smooth the data too much causing a possible loss of information. The first slope was calculated between minutes one and three; the next slope is between minutes two and four. This one-minute increase to acquire the next two-minute slope is repeated through the entire BRSI array. Figure 3.2 is a graph by group of the slopes for the full 56 minute HUT.

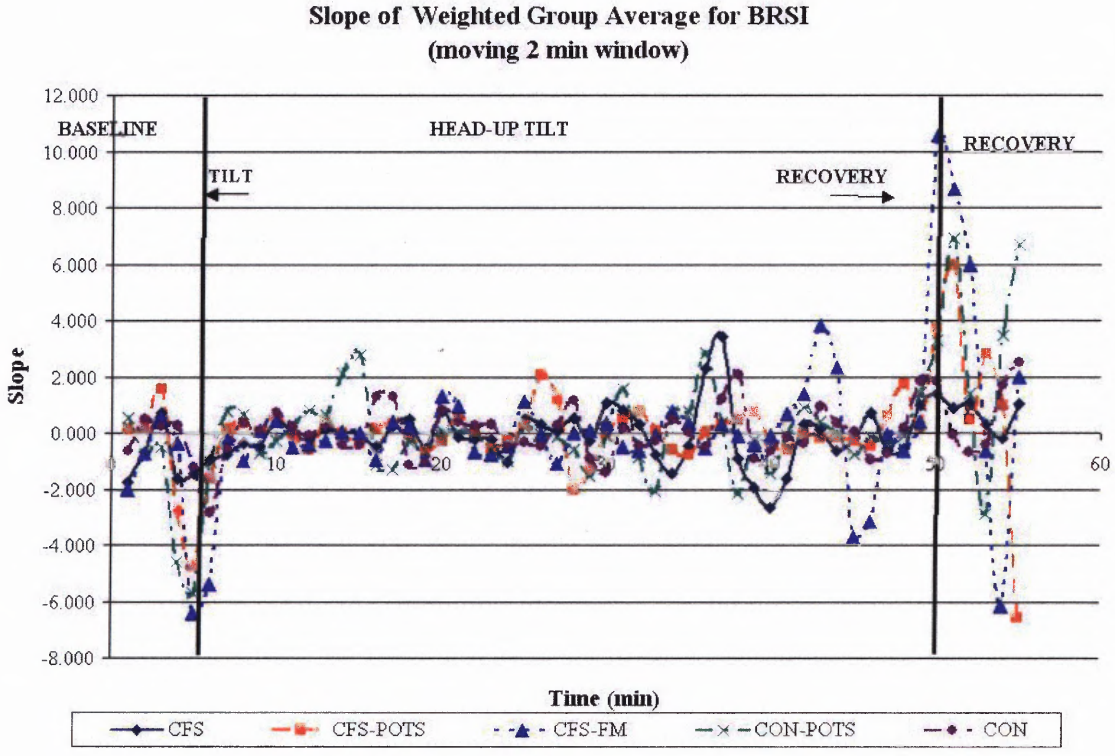


Figure 3.2 Graph of slopes for BRSI averaged values between groups.

The larger spikes for CON-POTS, CFS and CFS-FM groups during tilt is a result of the subjects within the respective group that experienced presyncopal or syncopal

symptoms and, as a result had their tilt ended. Slopes during recovery for CFS-FM, CFS-POTS and CON-POTS have a sharper transition in slope between tilt and recovery. This is consistent with the results seen in Figure 3.1. It may be possible based on these limited results to use slope and BRSI weighted averages during the tilt and recovery transition to identify CFS-FM subjects.

3.3 Analysis of BRSI by Lag Over Time

Figure 3.3 is a graph of weighted average for BRSI of lag 0 for all groups. The same trend observed in Figure 3.1 is present for lag 0. Stronger slopes for lag 0 are the most noticeable difference between Figure 3.3 and Figure 3.1.

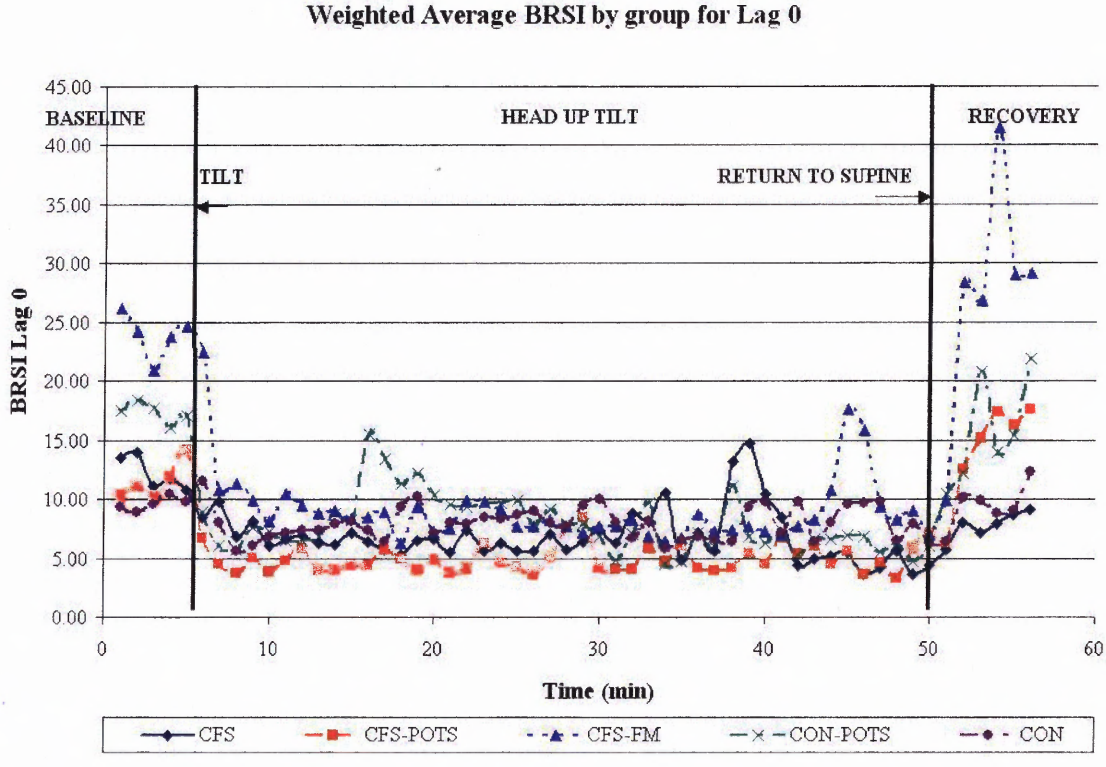


Figure 3.3 Graph of Lag 0 BRSI values by group.

In Figure 3.3, the spikes are a little sharper with the amplitudes slightly higher as opposed to Figure 3.1. The trends for lag 0 appear identical to those of Figure 3.1. CFS-FM again has the highest amplitudes during baseline and recovery. Figure 3.3 is sectioned to indicate the three phases of the test. Baseline, head-up tilt (HUT) and recovery are indicated. As was evident in Figure 3.1 at the transition between baseline and tilt and again between tilt and recovery a noticeable change in the baroreflex sensitivity index, BRSI, is present for all groups. The graph of weighted BRSI values for lag 1 is shown in Figure 3.4.

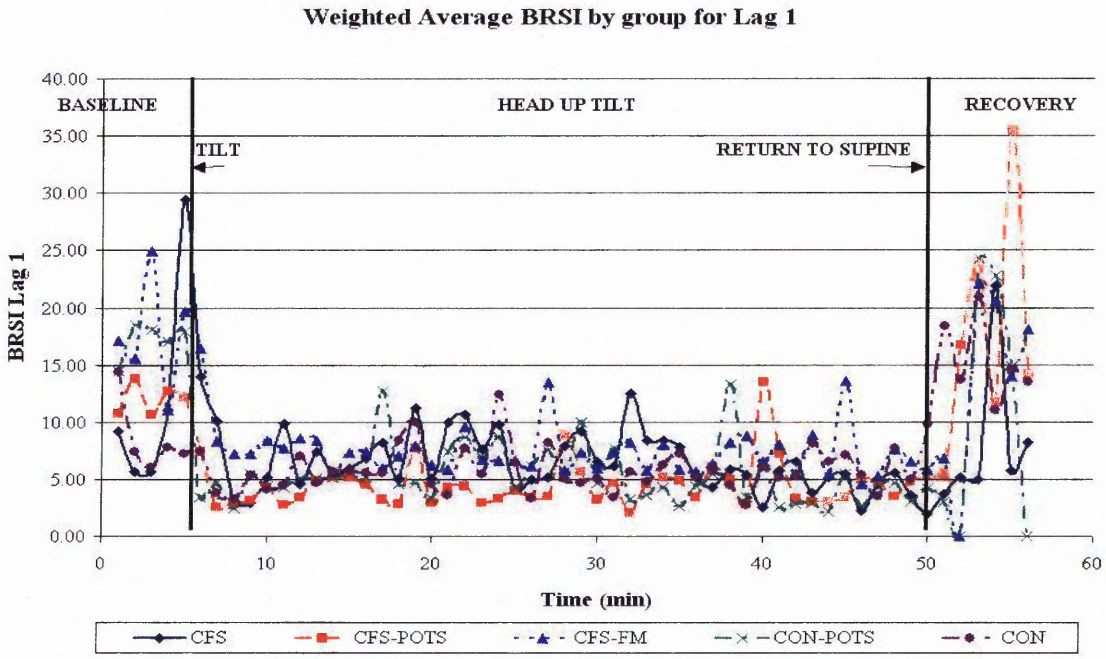


Figure 3.4 Graph of Lag 1 BRSI values by group.

A higher degree of variability of BRSI values for the entire time course is the first observation for lag 1. During baseline, the BRSI value for CFS increases dramatically from the levels seen previously. It is no longer possible to distinguish CFS-FM from the

from the levels seen previously. It is no longer possible to distinguish CFS-FM from the other four groups as the amplitudes for BRSI for this group have dropped dramatically. During recovery, all groups spike upward with CFS-POTS experiencing the most noticeable rise. The spikes, which indicated when subjects within a group experienced syncope, can no longer be discriminated from the random variability during the head-up tilt phase of the test.

There were fewer lag 2 sequences in general for all groups; lag 2 graph is shown in Figure 3.5. Graphically, similar variability exists for lag 2 as in lag 1 during tilt. The

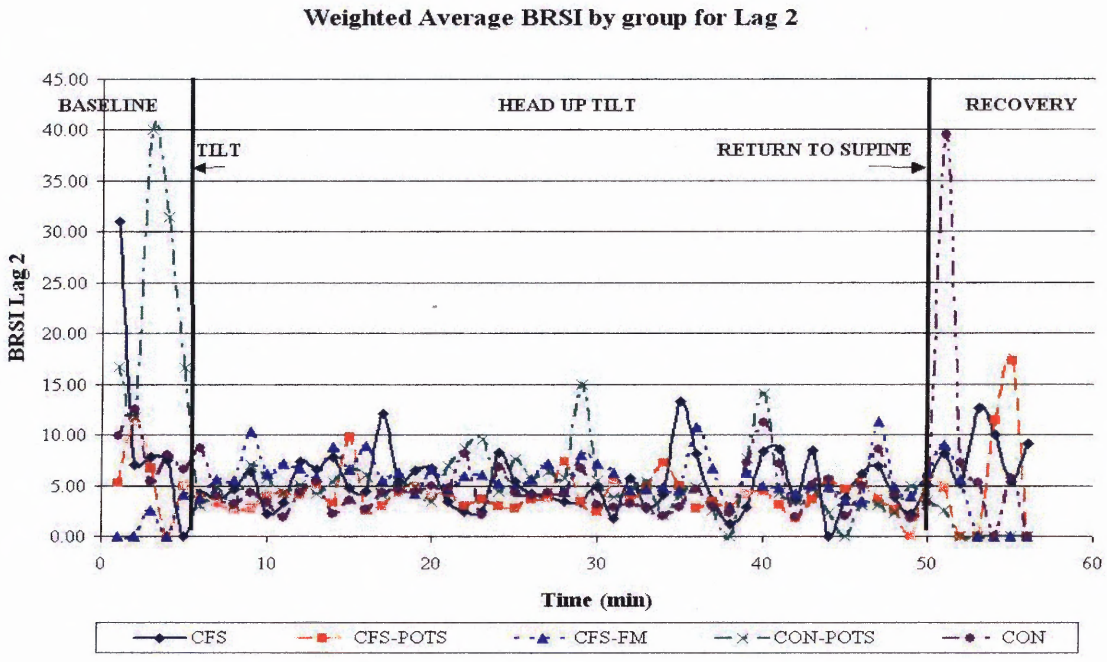


Figure 3.5 Graph of BRSI for Lag 2 for all groups.

large spike for CFS during baseline is similar to the one present during lag 1 however, it occurs earlier in lag 2. The CON-POTS group, for the first time, has a larger spike during baseline, followed closely by CFS. It is still not possible to identify the groups

that lost subjects due to syncopal or presyncopal symptoms. Immediately after tilt has ended the CON group spikes upward considerably, and this is the largest BRSI value for CON for all lags. Although the groups are small, statistical analysis may provide additional information as to the variability between intervals and groups.

3.4 Analysis of Ramps and Sequences Over Time

The graph of total number of ramps over time by group is shown in Figure 3.6. The graph of the total number of sequences over time by group is shown in Figure 3.7. Although the numbers of ramps and sequences may appear comparable, it is important to note the vertical scale. The y-axis for ramps begins at zero and increments by 20 at each tick mark. The y-axis for sequences begins at zero but increments by 10 at each mark.

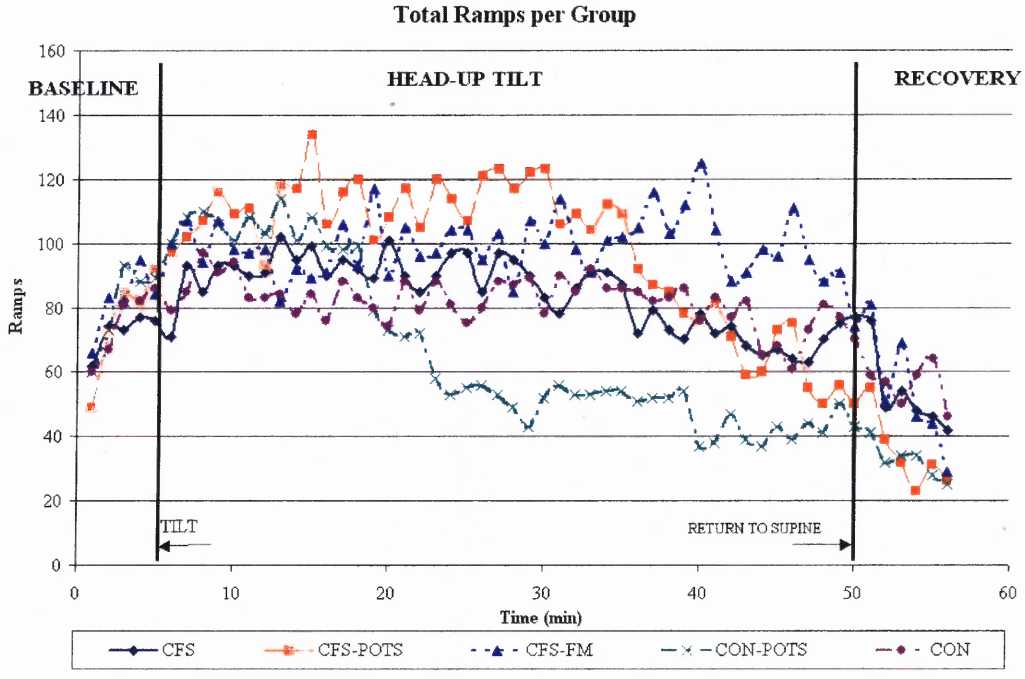


Figure 3.6 Graphs of total ramps by group over time.

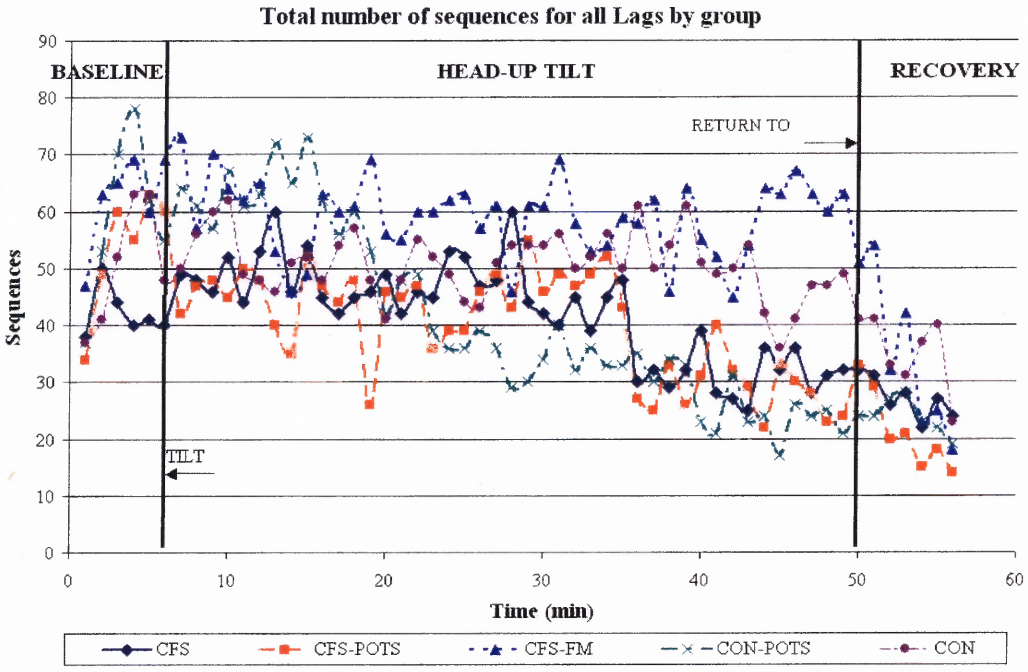


Figure 3.7 Graphs of total sequences by group over time.

Graphs of ramps and sequences broken down into beginning, middle and end of HUT are shown in Appendix C. The numbers in each graph represent the total number of ramps and sequences for that time interval. The total number of ramps per group per interval is lower during baseline but increases at tilt. The total number of ramps decline slowly during the course of tilt with the exception of the CON-POTS group whose numbers decrease more dramatically. The larger decrease in total number of ramps for CON-POTS is due to the loss of data for two subjects with presyncopal or syncopal symptoms. The decline in ramps for all groups continues into the six-minute recovery period.

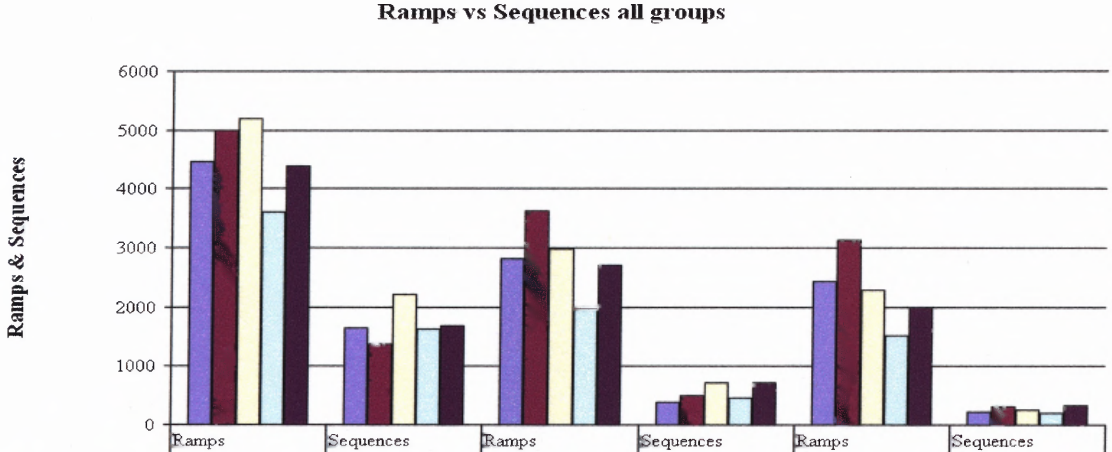
At baseline, the total number of sequences is greater than during tilt. At tilt, the total number of sequences begins a decline that continues through the six-minute

recovery period. The decline in sequences is more pronounced than the decline in ramps over the same time course. These graphs agree with the brief results shown in Section 3.1. Sequences cannot exist without ramps so it is logical that the number of ramps would be greater than the number of sequences. However, when a lower number of ramps are available at baseline the number of sequences occurring from them is greater. The ratio of ramps to sequences is discussed in Section 3.6.

Section 3.5 illustrates the ramp and sequence numbers a little differently by totaling them for lag by group and using a bar graph and table to illustrate the differences. Figures 3.6 and 3.7 illustrate minute-by-minute changes in both ramps and sequences.

3.5 Total Ramps and Sequences by Lag per Group

Ramps and sequences for each lag were totaled by group and are shown in Figure 3.8.



	LAG 0		LAG 1		LAG 2	
	Ramps	Sequences	Ramps	Sequences	Ramps	Sequences
CFS	4465	1650	2815	385	2430	220
CFS-POTS	4997	1370	3627	501	3126	306
CFS-FM	5190	2206	2984	705	2279	250
CON-POTS	3603	1637	1966	449	1517	209
CON	4391	1692	2699	713	1986	327

Figure 3.8 Bar graph of total ramps and sequences by group per lag.

The bar graph is separated into groups by lags for both ramps and sequences. A table, which is part of the graph, indicates the total number of ramps and sequences. The data in Figure 3.8 is the same data shown previously in Figures 3.6 and 3.7. It is presented differently in order to illustrate the use of ramps by the various lags and to visualize the number of sequences produced by each lag.

Table 3.2 indicates the percentage of sequences produced from the available ramps for each lag within each group, or a BEI value by lag. The most efficient use of ramps is by lag 0 for all groups. The number of sequences tapers off dramatically with larger lags regardless of the availability of ramps and is consistent between groups in that they all decrease appreciably from lag 0. Table 3.2 lists the total number of sequences produced and the available ramps for each lag as well as the percentage of ramps used based on sequence production. When a ramp produces a sequence in a lag it is removed from the pool of available ramps for subsequent lags. The ramp value listed for lag 1 is the ramp value from lag 0 minus the sequence value from lag 0. The ramp value for lag 2 is the ramp value from lag 1 minus the sequence value from lag 1.

Table 3.2 Baroreflex effectiveness index by lag.

	Lag 0			Lag 1			Lag 2		
Group	Seq.	Ramps	%	Seq.	Ramps	%	Seq.	Ramps	%
CFS	1650	4465	37.0%	385	2815	13.7%	220	2430	9.1%
CFS-POTS	1370	4997	27.4%	501	3627	13.8%	306	3126	9.8%
CFS-FM	2206	5190	42.5%	705	2984	23.6%	250	2279	11.0%
CON-POTS	1637	3603	45.4%	449	1966	22.8%	209	1517	13.8%
CON	1692	4391	38.5%	713	2699	26.4%	327	1986	16.5%

Table 3.3 shows the percent decrease in the number of sequences between lags for each group. Only one study has been identified that addressed the percent decrease of sequences [33]. This study by Bertinieri et al. evaluated baroreceptor response in cats before and after sinoatrial denervation. Sinoatrial denervation severs the baroreceptor removing the input to the cardiovascular control center, thus removing the baroreceptor afferents control of heart rate. The reported decline in sequences from this study was not separated by lag rather it was an overall decrease.

In Table 3.3 the lower the percentage the less variable the group is in the number of sequences generated from available ramps across lags. The two most consistent groups in the number of sequences generated from the available ramps across all lags are CON and CFS-FM. The least consistent group is CFS.

Table 3.3 Percent decrease in sequence production between lags.

GROUP	lag 0 - lag 1	lag 1 - lag 2	lag 0 - lag 2
CFS	76.7%	42.9%	86.7%
CFS-POTS	63.4%	38.9%	77.7%
CFS-FM	68.0%	64.5%	88.7%
CON-POTS	72.6%	53.5%	87.2%
CON	57.9%	54.1%	80.7%

3.6 Analysis of BEI by Group Over Time

An analysis of baroreflex effectiveness index (BEI) overtime was done in an attempt to understand what if any additional information it provides. Figure 3.9 is an overall graph of BEI by group per one-minute interval for the entire tilt. Figure 3.10 is a graphical representation of the percentages shown in Table 3.2.

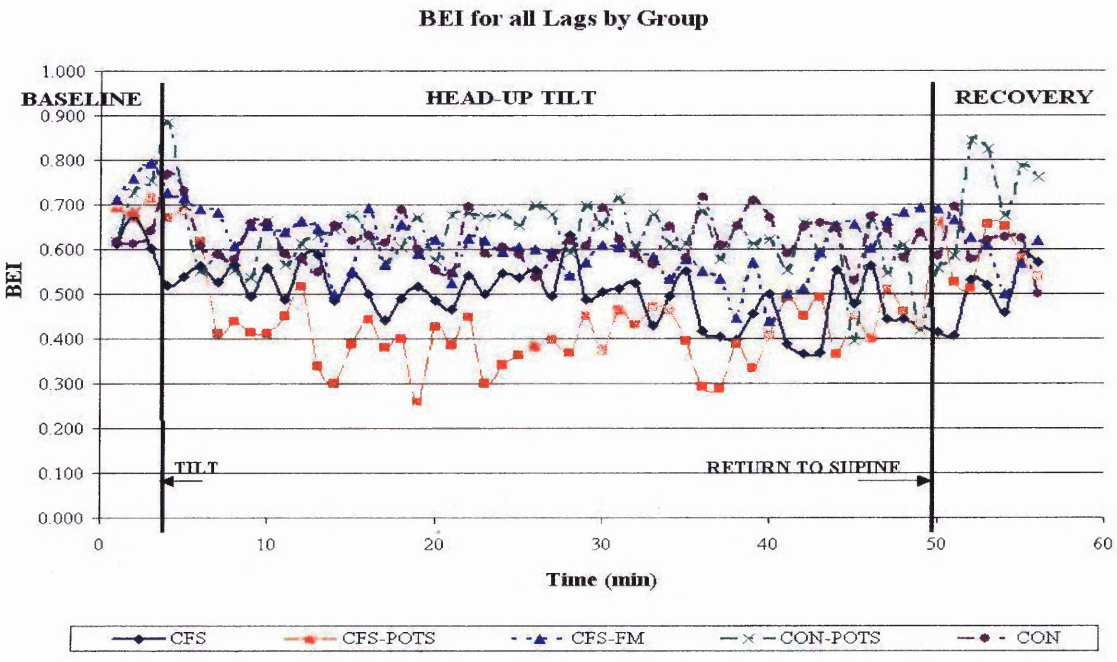


Figure 3.9 Graph of BEI for all groups for full test.

The BEI results shown in Figure 3.9 were calculated by taking the total number of sequences for each group for all lags and dividing this sum by the total number of ramps, regardless of whether or not they produced a sequence. The BEI values in Figure 3.9 follow the same trends present when observing average BRSI by group in Figure 3.1.

BEI is an indication of the baroreflex effectiveness, or the number of times the baroreflex is effective in its drive to control heart rate based on spontaneous SBP input.

In Figure 3.9 BEI is higher during transitions from baseline to tilt and again from tilt to recovery. During tilt, BEI is lower for all groups, particularly CFS-POTS.

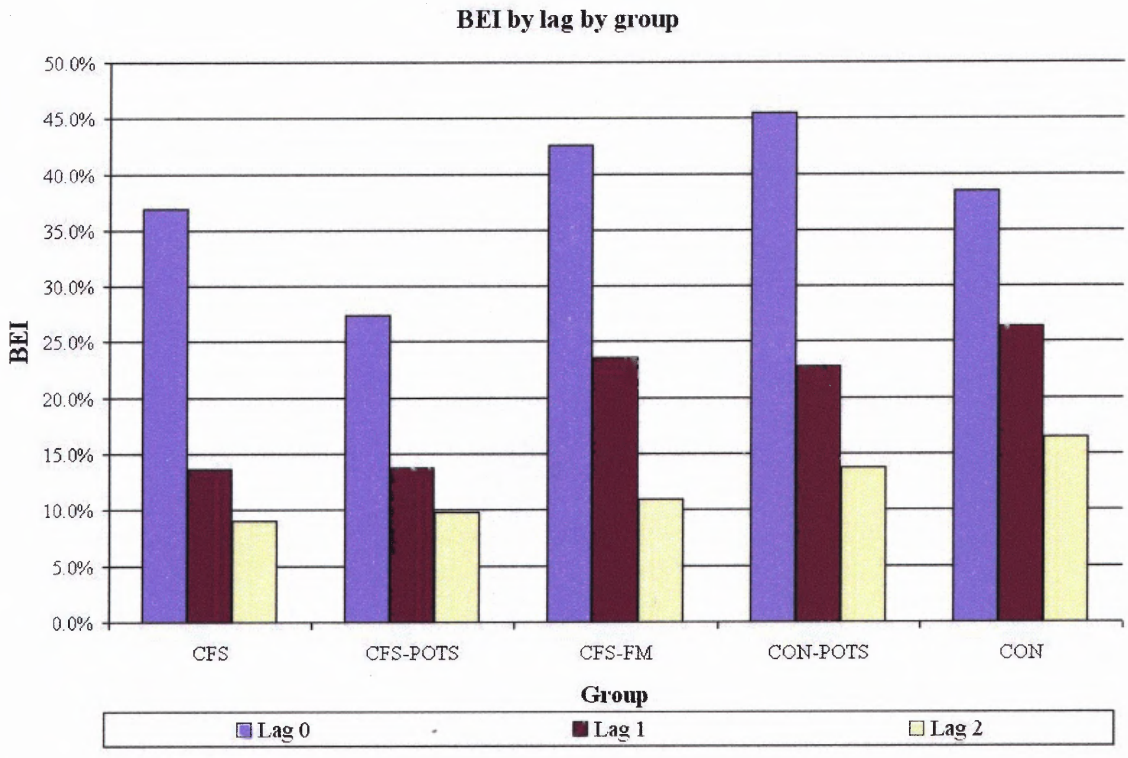


Figure 3.10 BEI per group by lag.

Figure 3.10 shows the comparison between lags for each group. BEI decreases as the lag number increases. CON-POTS followed by CFS-FM have the highest BEI values of all groups. CON produces the largest number of sequences for lags 1 and 2 of all groups. The use of BEI as a measure of the number of times the baroreflex is effective may aid in characterizing these disease states. Supplemental graphs that break down Figure 3.9 into beginning, middle and end sections are shown in Appendix C.

CHAPTER 4

CONCLUSIONS

Although numerous studies evaluate baroreflex response as part of their research protocol, only one was identified as having evaluated continuous baroreceptor response as part of a head-up tilt. This study done by Youde et al. [28] evaluated continuous baroreceptor measurement in healthy elderly individuals. Each subject, on two separate occasions, underwent three 70^0 head-up tilts that lasted for 10 minutes each. Sequences in that study were defined as three beats exclusively, in addition, BRS was only evaluated for lag 0. The limitations of the Youde study [28] are not present in this thesis.

For this thesis, head-up tilt was also 70^0, however it lasted for 45 minutes unless the subject exhibited presyncopal or syncopal symptoms. All data was gathered in one visit, sequences were defined as three or more beats and data for lags 0, 1 and 2 were evaluated. The sequence method was used to acquire a baroreflex sensitivity index, BRSI, to assess baroreflex function. Comparative time analyses were done of weighted BRSI values between groups as well as between lags within groups. Baroreflex effectiveness index, BEI, was also evaluated along with the total number of ramps and sequences within and between groups.

Comparative time analysis provided a graphical representation of the behavior of the baroreflex during head-up tilt. In addition, a comparison between groups of weighted BRSI values graphically shows the differences in BRSI values between subject groups. The most obvious differences occur during baseline, when the subjects are supine and again during recovery when tilt has ended. Graphically it appears as though, of the five

groups analyzed, a preliminary distinction could be made for CFS-FM and possibly CON-POTS based on these results. A statistical analysis utilizing repeated measures ANOVA and paired t-tests would provide the information necessary to determine the significance between the groups. Evaluation of the slope of BRSI over the course of head-up tilt does not appear graphically to be a marker to discriminate between subject populations with the possible exception of CFS-FM.

Evaluation of BRSI by lag was done for this thesis. This type of evaluation was not identified in the literature researched as having been performed previously. Lag 0 appears to mimic the group trends with subtle differences in amplitude. The graph of the group results for lag 1 was noticeably more variable than the graphs of the group results with combined lags the lag 0 group results. Graphically lag 2 shows large spikes during baseline for CFS and CON-POTS. During recovery, the CON group spikes sharply with the end of tilt.

Analysis of BEI graphically has shown similar trends to the graphs of weighted BRSI averages. BEI is an indication of the baroreflex effectiveness, or the number of times the baroreflex is effective in its drive to control heart rate based on spontaneous SBP input. It is not clear from the results whether BEI is providing any additional information. Because of the way BEI is calculated it a convenient measure to include when using the sequence technique to analyze BRSI. It seems logical to continue using BEI in the event that its utility becomes more apparent. Further, a comparative time analysis of the number of sequences versus the number of available ramps shows what appears to be a comparable trend for all groups. However, when a table representing the percent change between lags is done, the two most consistent groups in number of

sequences produced from available ramps across lags are CON and CFS-FM with the least consistent being CFS. Statistical analysis is necessary to evaluate this seemingly large difference.

The utilization of analysis over time to evaluate BRSI is an unexplored area of baroreflex research. Based on the results of this thesis the idea appears to have merit. Analysis of BRSI over time may prove to be an additional tool to aid in the discrimination of patient groups. At the very least, this type of analysis could provide additional information in the understanding of baroreflex function in controlling heart rate. It is reasonable to consider the use of head-up tilt tests in conjunction with BRSI and BEI analysis to distinguish between CFS subject groups.

Statistically repeated measures ANOVA analysis for should be performed to determine the utility of comparative time analysis of BRSI in the evaluation of CFS through the course of a head-up tilt. Single factor ANOVA does not do this; it determines only if there is a difference in the mean between the groups and does not consider the changes by time intervals between or within groups. In order to understand the changes over time repeated measures ANOVA needs to be done. This would lead to post hoc analysis, which would further delineate between the subject groups.

Given the small sample size, it would be prudent to expand the database prior to attempting to determine significance, statistically or graphically.

4.1 Future Work

The efficacy of the interpolator needs to be tested. In order to do this data with no gaps in the SBP interval array, should be run through the interpolator and sequencer programs with the results written to an Excel file. The data should then have gaps placed in the SBP data and saved under another file name. This new data with holes inserted, should be run through the interpolator and sequencer programs with the results saved to another Excel file. The two Excel files need to be compared to evaluate the utility of the interpolator. If results of the interpolator tests are positive it would allow future studies that utilize the Finapres™ to enable the calibration circuit throughout data acquisition. The interpolator program was written specifically for the LaManca study [3] data that was analyzed for this thesis. If tests of the interpolator validate its use, it needs to be updated to run any data.

There has been no research found to date that addresses the evaluation of diastolic blood pressure. Only one was found [17] that attempts to address non-baroreflex sequences however, this was not a head-up tilt test analysis. Improvements to the sequencer to include these analyses could provide additional information not presently available or actively researched.

The sequencer programming should be adjusted to separate positive and negative sequences. This would allow for the evaluation of each separately to determine if one or the other is a more effective baroreflex response. Di Rienzo et al. [13] in their BEI studies have presented their BEI findings separately for positive and negative sequences. This change would make the results of the sequence program comparable to current research.

This thesis only considers sequences of three or more beats. The sequencer program currently has the capability for the user to input a number defining the minimum number of beats that are necessary for a sequence. Four beat sequence analysis could be done and compared to the current analysis to identify what if any additional information could be gained for this or other data sets.

The eliminator program should be incorporated into the sequencer as a subroutine. At present, the eliminator is a stand-alone program that needs to be run prior to sequencing any data to remove editing errors. Inclusion of the eliminator program as a subroutine in the sequencer program would streamline the process. The eliminator program does not alter data if no editing errors are present. The original data is passed through intact.

Presently in the exclusive subroutine if more than one sequence is produced by the last ramp the sequence that occurs subsequent to the first is not eliminated. This problem should be corrected.

APPENDIX A

SUBJECT INFORMATION

The table below identifies the data, in minutes, for each subject by group for baseline, tilt and recovery. The duration of a complete head-up-tilt test was 56 minutes including baseline and recovery . Shorter duration tests indicate the subject experienced syncopal or presyncopal symptoms.

Subject	Group	Baseline (min)	Tilt (min)	Recovery (min)	Total (min)
t125	CFS	5	30	6	41
t182	CFS	5	24	6	35
t211	CFS	5	45	6	56
t214	CFS	5	45	6	56
t235	CFS	5	45	6	56
t131	CFS-POTS	5	32	6	43
t146	CFS-POTS	5	45	6	56
t193	CFS-POTS	5	45	6	56
t203	CFS-POTS	5	44	4	53
t206	CFS-POTS	5	22	8	35
t207	CFS-FM	5	43	5	53
t231	CFS-FM	5	37	5	47
t244	CFS-FM	5	45	6	56
t248	CFS-FM	5	45	6	56
t257	CFS-FM	5	45	6	56
t335	CON	5	45	6	56
t358	CON	5	45	6	56
t363	CON	5	32	6	43
t385	CON	5	45	6	56
t399	CON	5	45	6	56
t312	CON-POTS	5	45	6	56
t375	CON-POTS	5	45	6	56
t380	CON-POTS	5	11	6	22
t391	CON-POTS	5	29	5	39
t392	CON-POTS	5	9	4	18

APPENDIX B

PROGRAM DESCRIPTIONS

The eliminator program as described in Section 2.2.1 is shown below in Figure B.1.

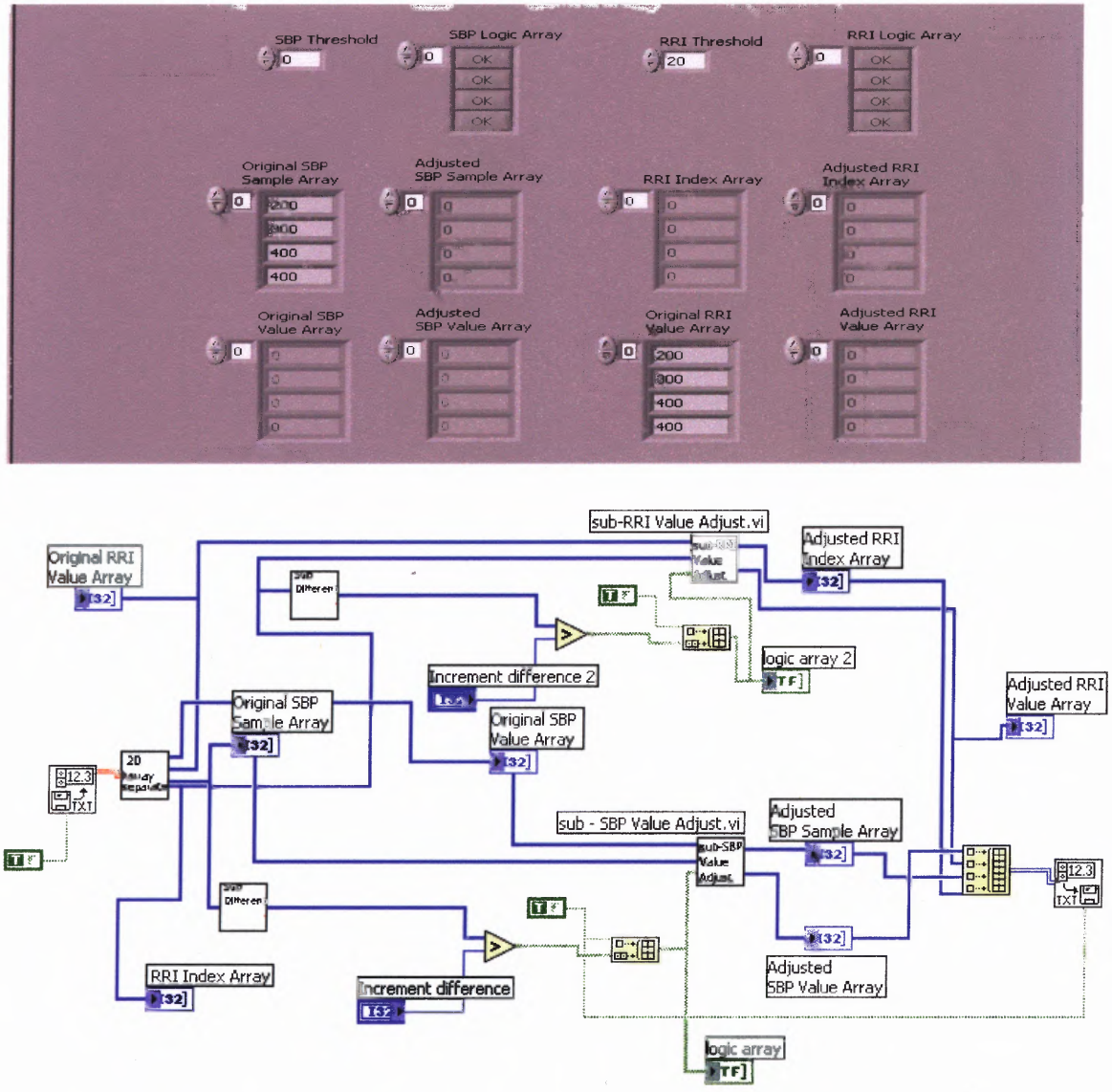


Figure B.1 Front panel (top) and block diagram (bottom) of the eliminator program.

The program reads in a four-column spreadsheet file, separates the four columns, calculates the difference array for the RRI and the SBP index arrays and compares the result to threshold values defined by the user. There are two threshold values one for each difference array. The comparative results are stored in true/false logic arrays that are used to eliminate the identified errors in another subroutine. Values are removed from the appropriate value and sample index arrays when errors are identified. Indices of editing errors were specific to either the RRI or SBP data. Figure B.2 is the front panel of the interpolator program.

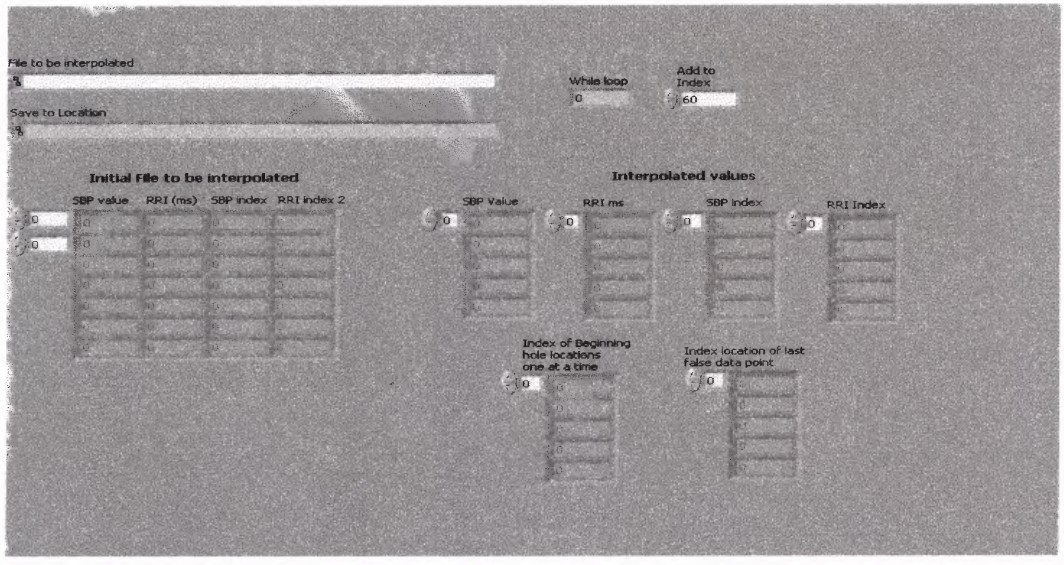


Figure B.2 Front panel of the interpolator program.

The file being interpolated and the save to location of the interpolated file are displayed in the top left of Figure B.2. The original spreadsheet file is displayed at the left along with the interpolated values on the right. The two arrays below the interpolated values store the index values for the gap locations. The control labeled, "Add to index", above right is used to interpolate the sample index arrays when a gap is located.

Figure B.3 is a block diagram of the interpolator program.

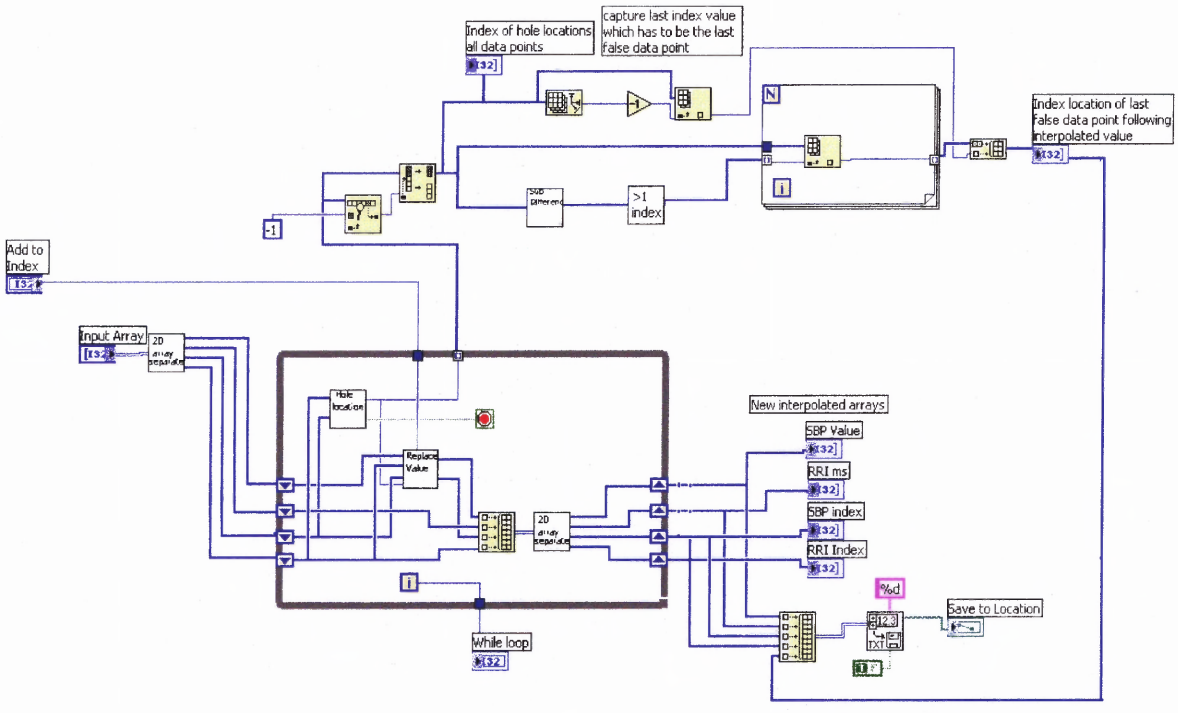


Figure B.3 Block diagram of interpolator.

A while loop is used to identify gap locations one point at a time. The index location of each gap is passed to another subroutine within the while loop that fills in the gap. Shift registers are used to build the new interpolated arrays as each gap is filled. The loops in the top of the diagram are capturing the last false data point or gap, which was filled for use by the sequencer program. The interpolated data along with the array containing the last false data point are written to file.

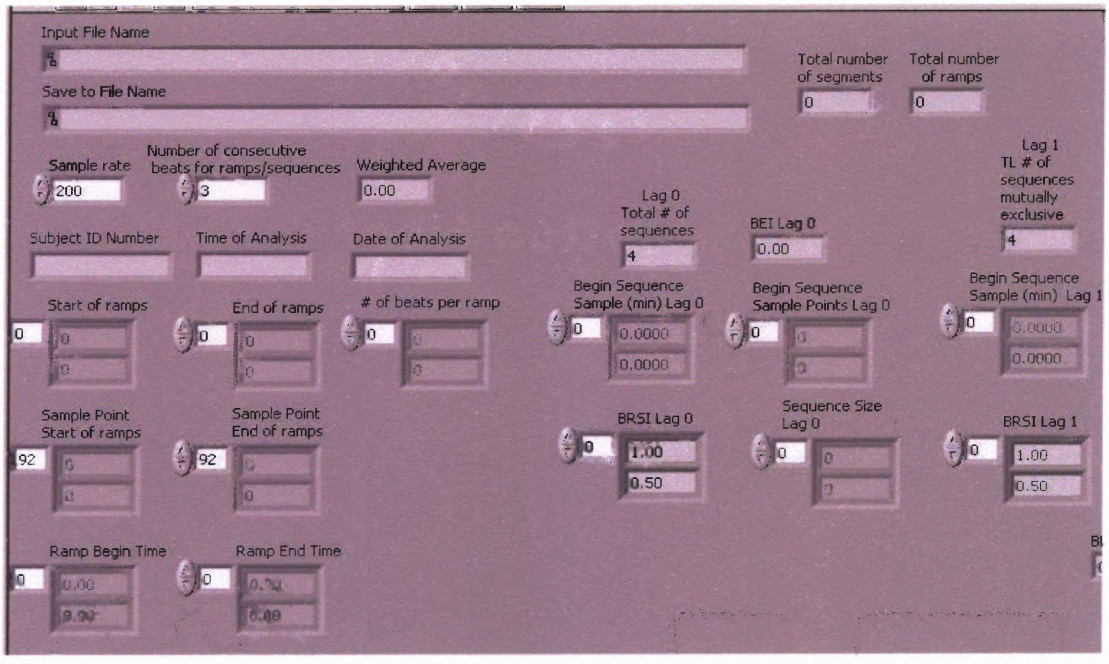


Figure B.4 Front panel of the sequencer.

There are three sequencers, each responsible for one lag. The lag 0 sequencer identifies segments and ramps for all sequencers as well as identifying valid sequences for lag 0. Ramps that are not used by the lag 0 are passed to the lag 1 sequencer that identifies lag 1 sequences. Ramps that are not used by the lag 1 sequencer are passed to the lag 2 sequencer to identify lag 2 sequences. The output of the program was described previously in Methods of Analysis. The final data for each lag are combined and written to file. A high-level view of the program behind the sequencer panel is shown in Figure B.5. The sequence process is described in Section 2.2.1.2.

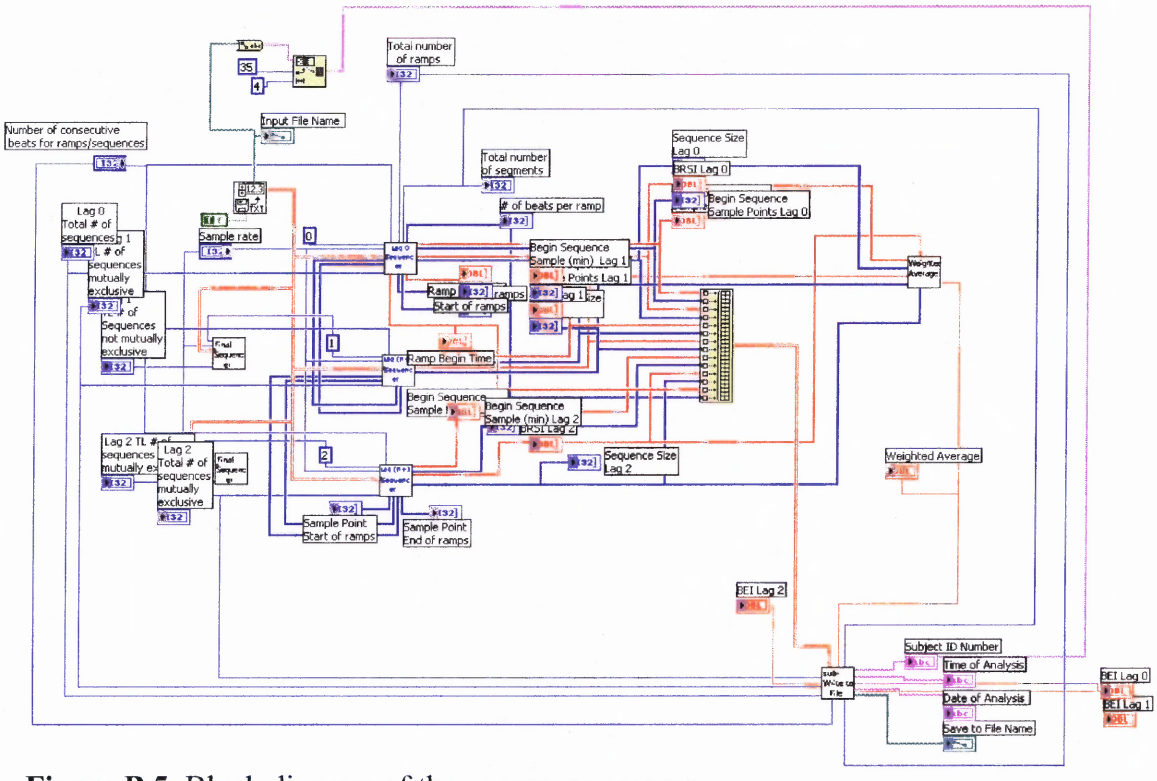


Figure B.5 Block diagram of the sequencer program.

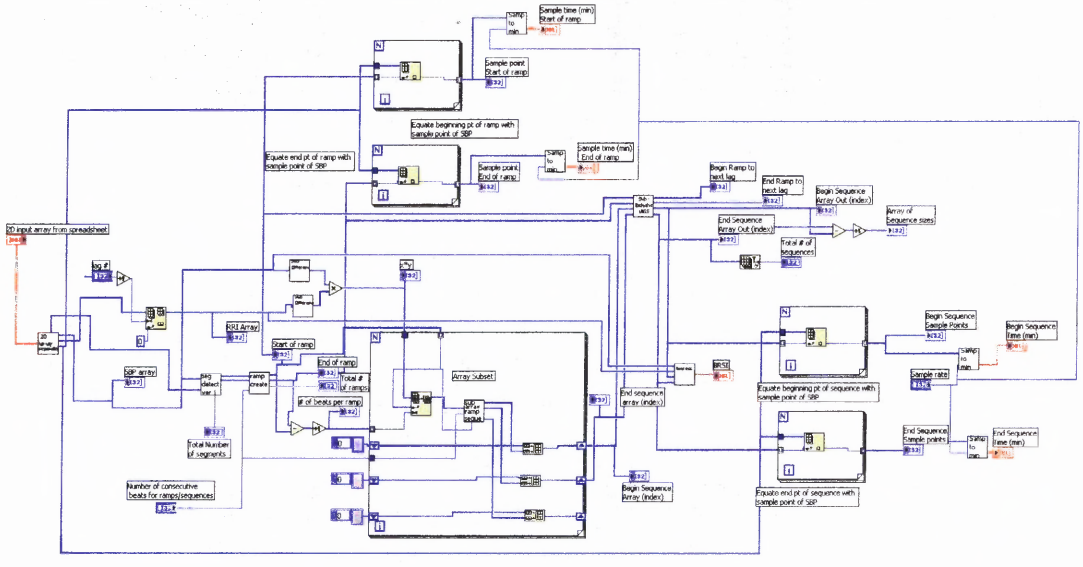


Figure B.6 Block diagram of lag 0 sequencer.

The block in the bottom center of Figure B.6 is a For loop. It uses several imbedded subroutines to identify valid sequences from ramps. The two blocks on the right are also For loops that equate the begin and end sequence index points to sample index values in order to calculate the begin and end times in minutes. The two For loops in the top of the diagram equate the ramp begin and end index values to sample indices to calculate the begin and end times for each ramp in minutes. Block diagrams for lags 1 and 2 are identical to Figure B.6 except that segment and ramp detection is done only in the lag 0 sequencer. Full sequence data along with ramp information is then written to file as described in Section 2.2.1.2.

It is possible for one ramp to produce more than one sequence as discussed in Section 2.2.1.2. The exclusive subroutine removes any sequence beyond the first that is produced from a single ramp. Figures B.7 and B.8 show the exclusive program front panel and block diagram respectively.

The begin sequence index is compared to the ramp indices, if the logic, shown in Figure B.8 is true the ramp is removed, if not the ramp is passed through and the comparisons to this sequence continue until the ramp that produced it is found. After the first sequence has been identified, the next sequence is compared to the same ramp, if the comparison is true the sequence is removed. This continues until all comparisons between ramps and sequences are complete.

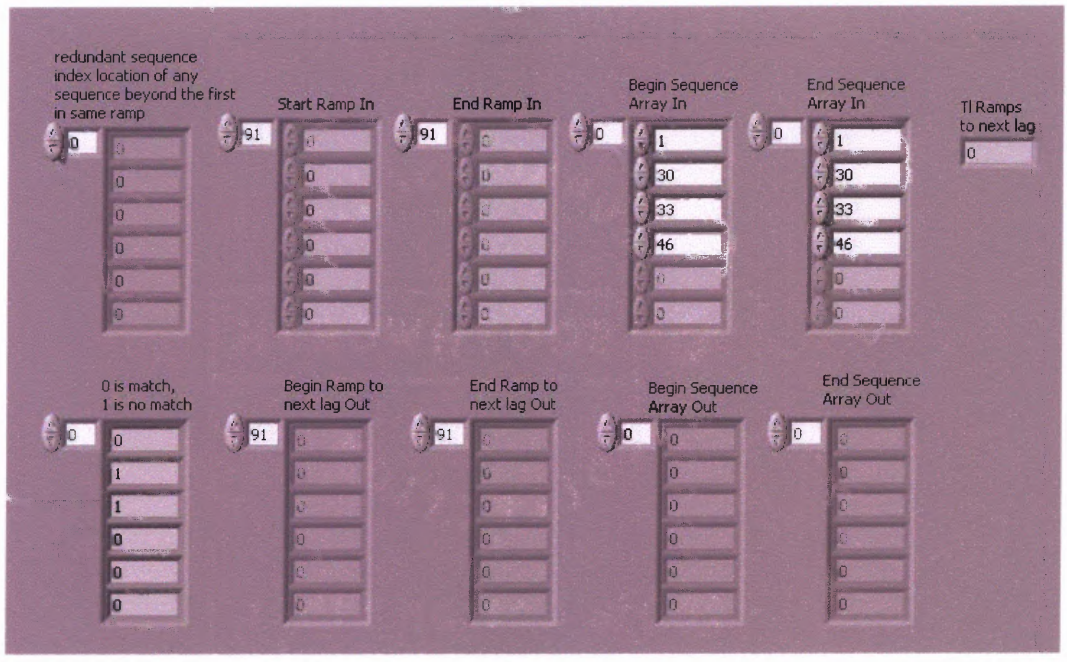


Figure B.7 The front panel of the exclusive program.

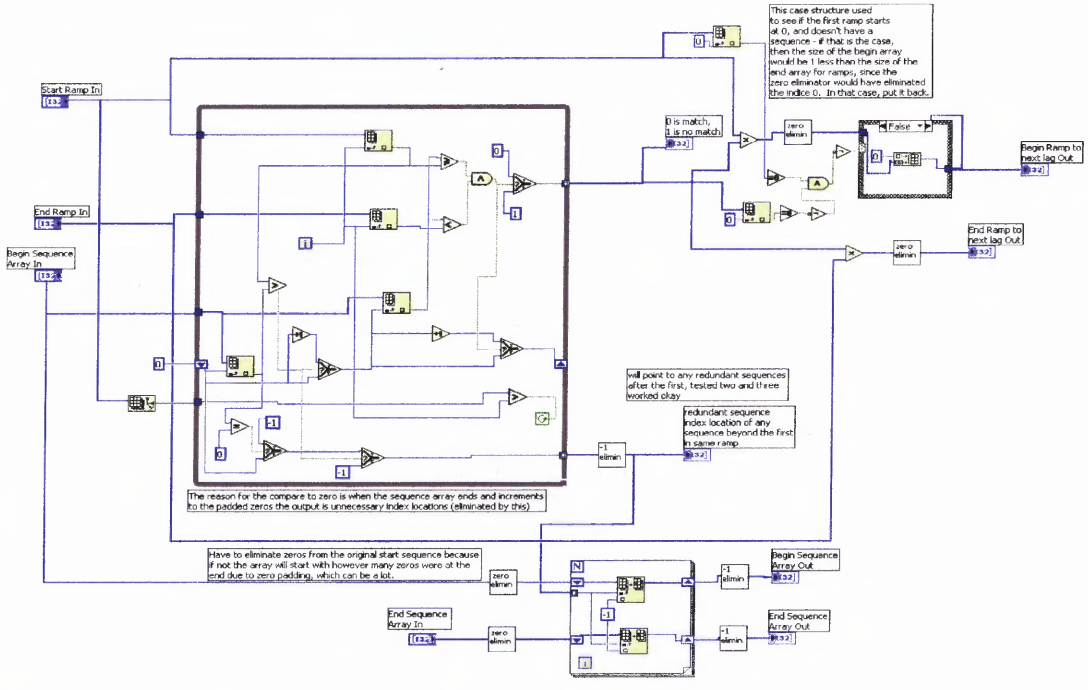


Figure B.8 Block diagram of the exclusive program.

The front panel of the analysis program is shown in Figure B.9. It indicates subject identification, time and date of analysis the path of the file being analyzed, save to file location, and all relevant analysis data. The control, "Group size in minutes", is user definable but must be an integer value. The program reads in a sequenced spreadsheet file.

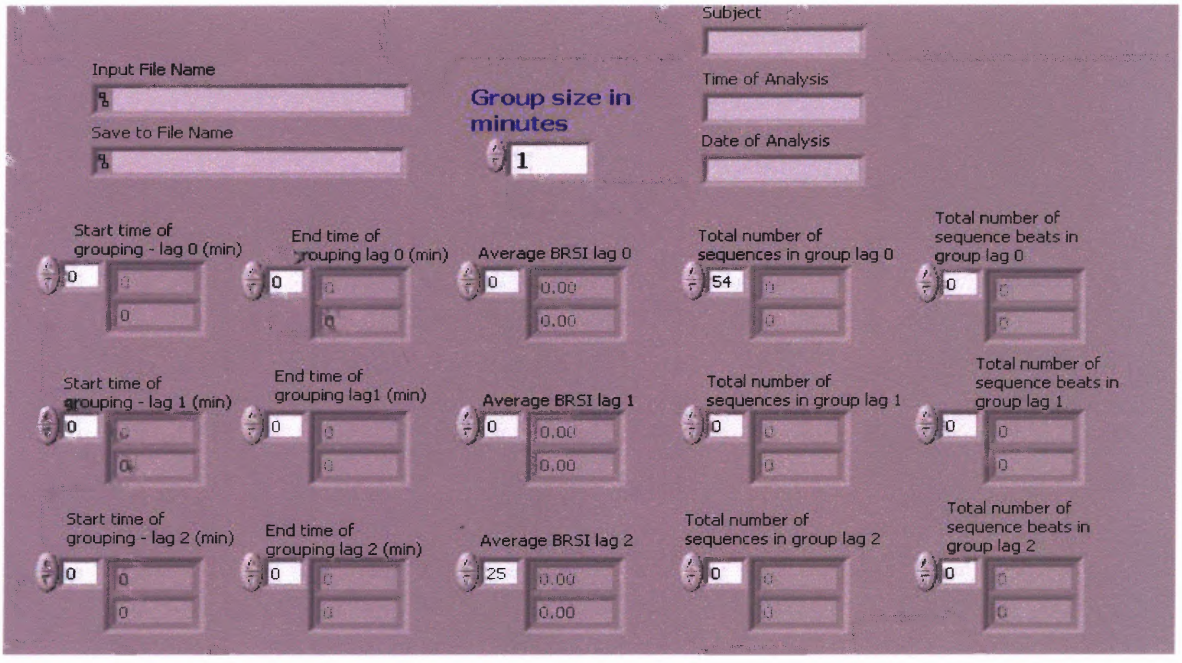


Figure B.9 Front panel of the analysis program.

The block diagram for the analysis program is shown in Figure B.10. On the left side of Figure B.10, the read from file information is shown. The file is separated into the various arrays required by the program. Three arrays for each lag, as described in Section 2.2.1.3 Analysis Programs, are passed to subroutines that separate the data based on the user defined group size. When all data has been processed, it is combined and

written to file along with header information titling each column. An example of the program output was shown in Figure 2.10.

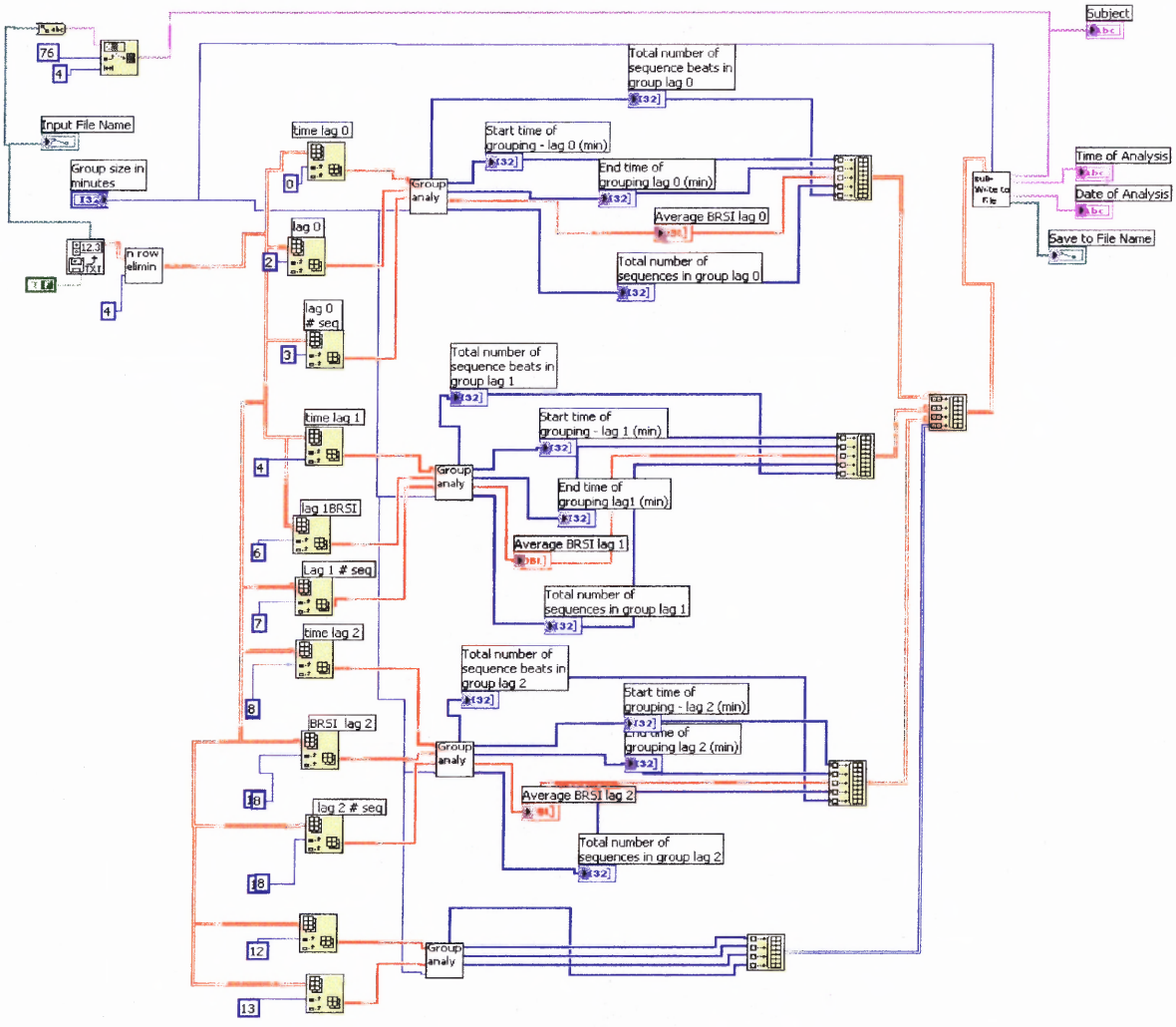


Figure B.10 Block diagram of the analysis program.

APPENDIX C

SUPPLEMENTAL GRAPHS

Figure C.1 is a graph of the first 15 minutes of tilt of the weighted average BRSI values by group for each one-minute interval. It includes a five-minute baseline period and the first 10 minutes of tilt. The graph is marked indicating when tilt occurs. The sharp drop in BRSI value is apparent when tilt occurs and was explained in Section 3.1.

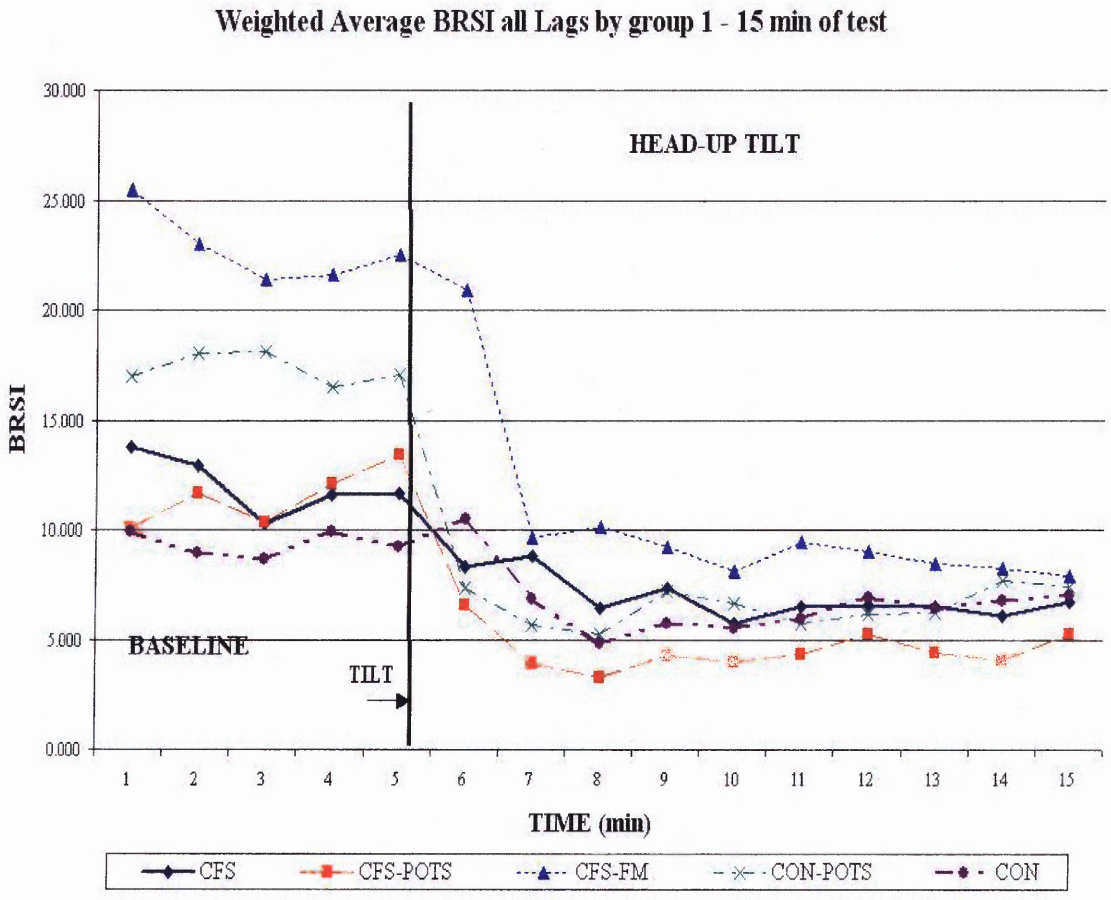


Figure C.1 Weighted average BRSI for first 15 minutes of HUT.

Figure C.2 is a graph of the remaining weighted average BRSI values by group for minutes 15 to 41 and 41 to 56 of HUT.

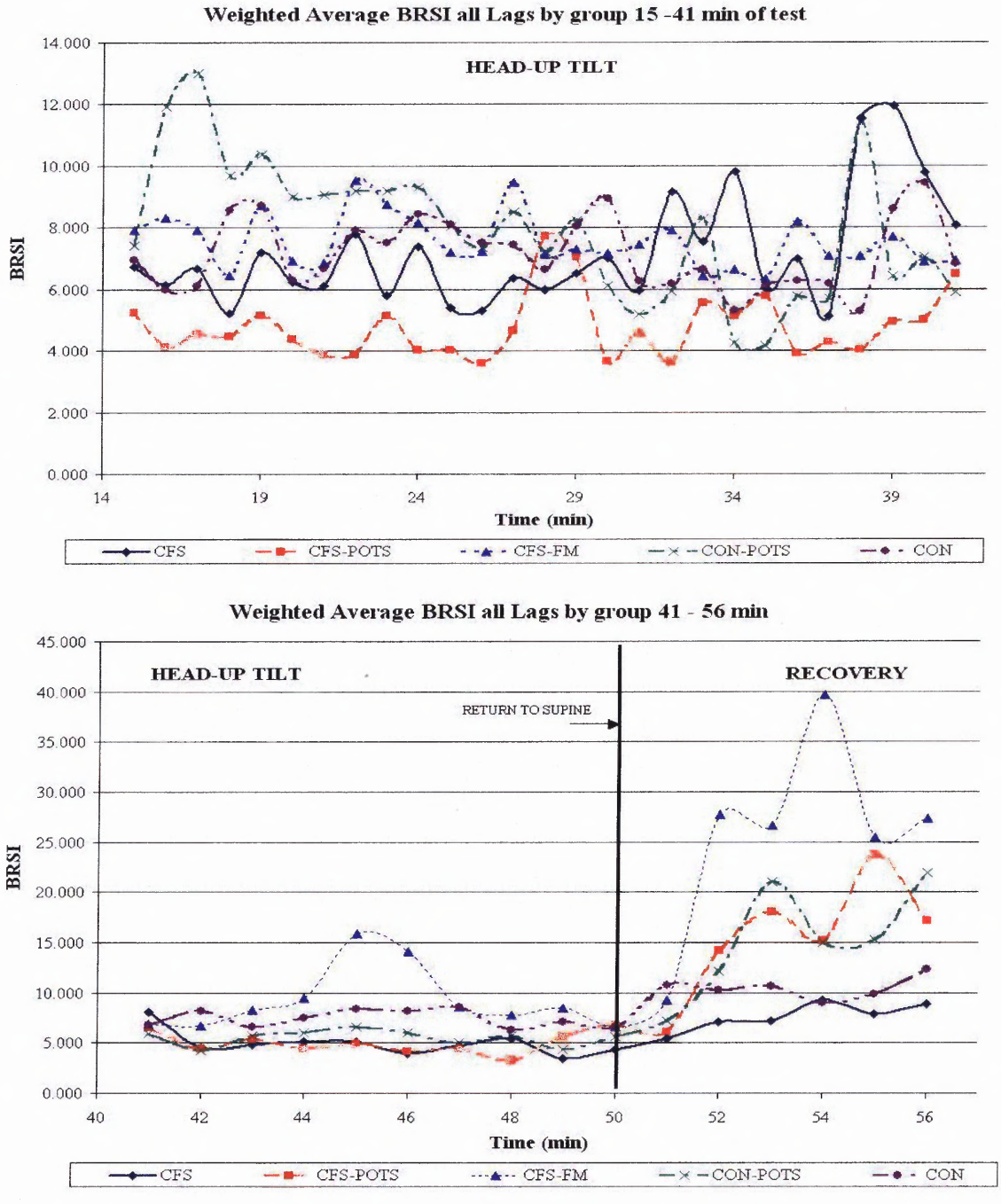


Figure C.2 Remaining weighted average BRSI values for 15 – 41 minutes and 41 – 56 minutes of HUT.

The graphs of ramps and sequences for the first 15 minutes of HUT are shown in Figure C.3. See Section 3.4 Comparative Time Analysis of Ramps and Sequences.

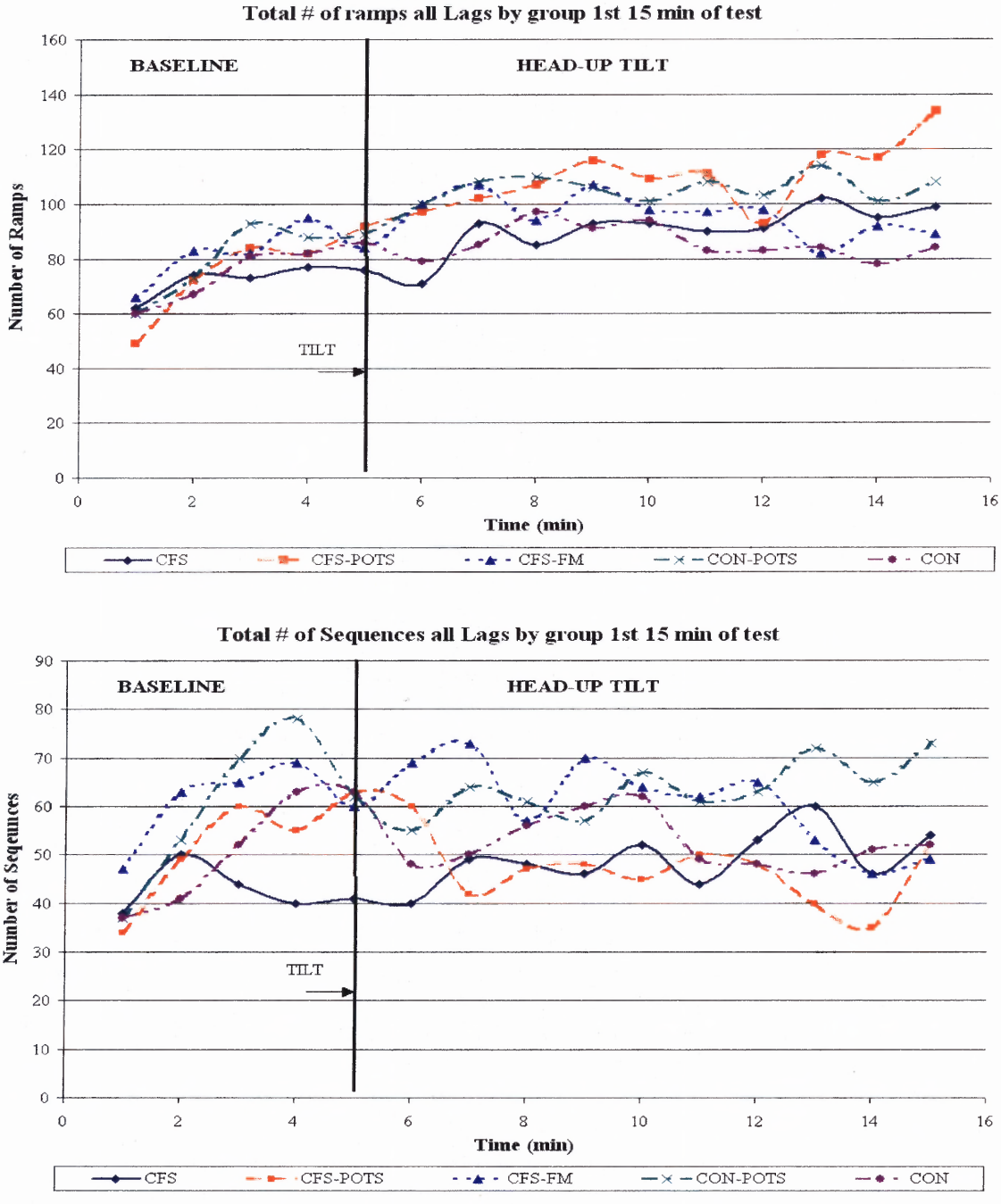


Figure C.3 Ramps and sequences for first 15 minutes of HUT. Reflects totals by group for each one-minute interval.

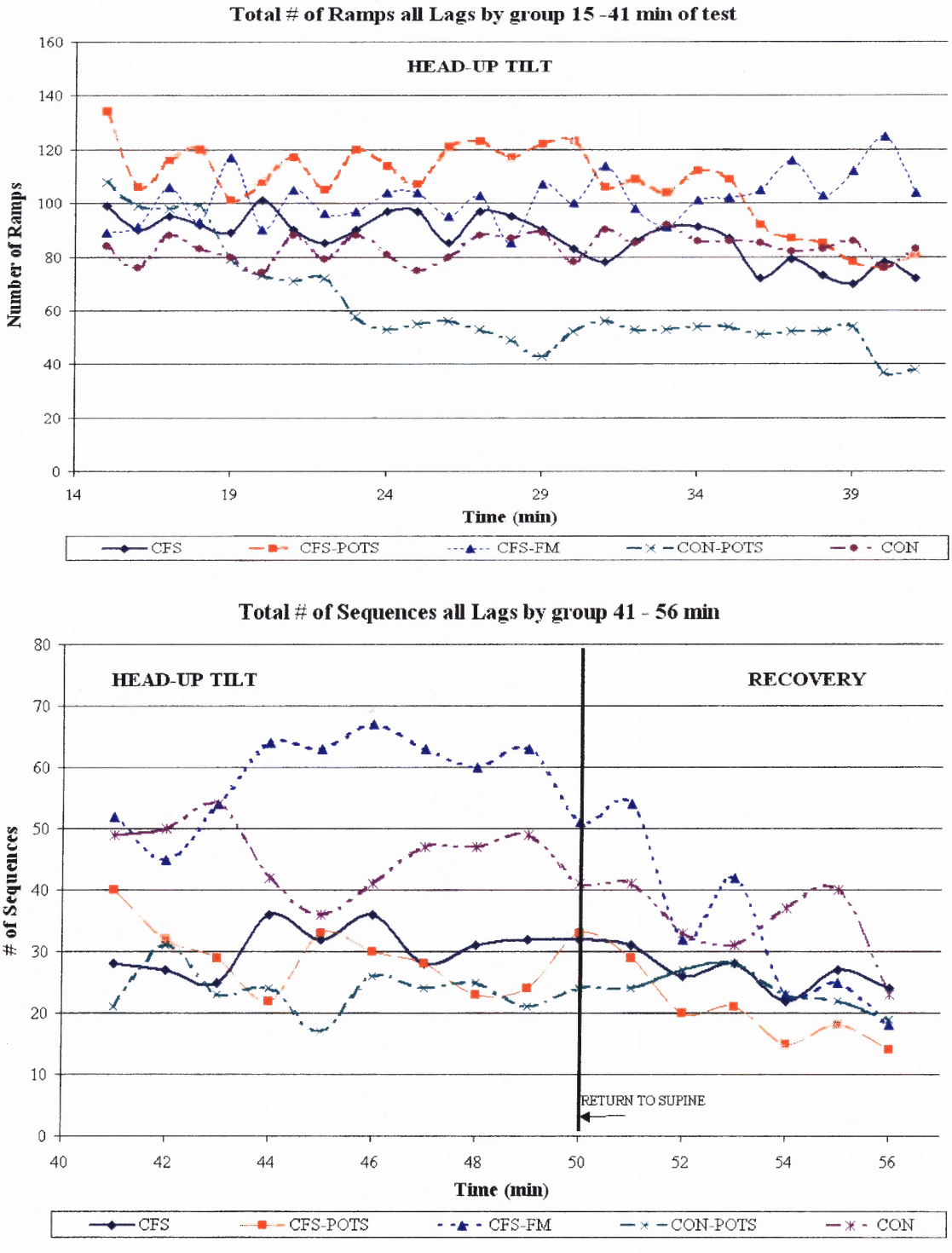


Figure C.4 Ramps and sequences for 15 - 41 minutes of HUT.

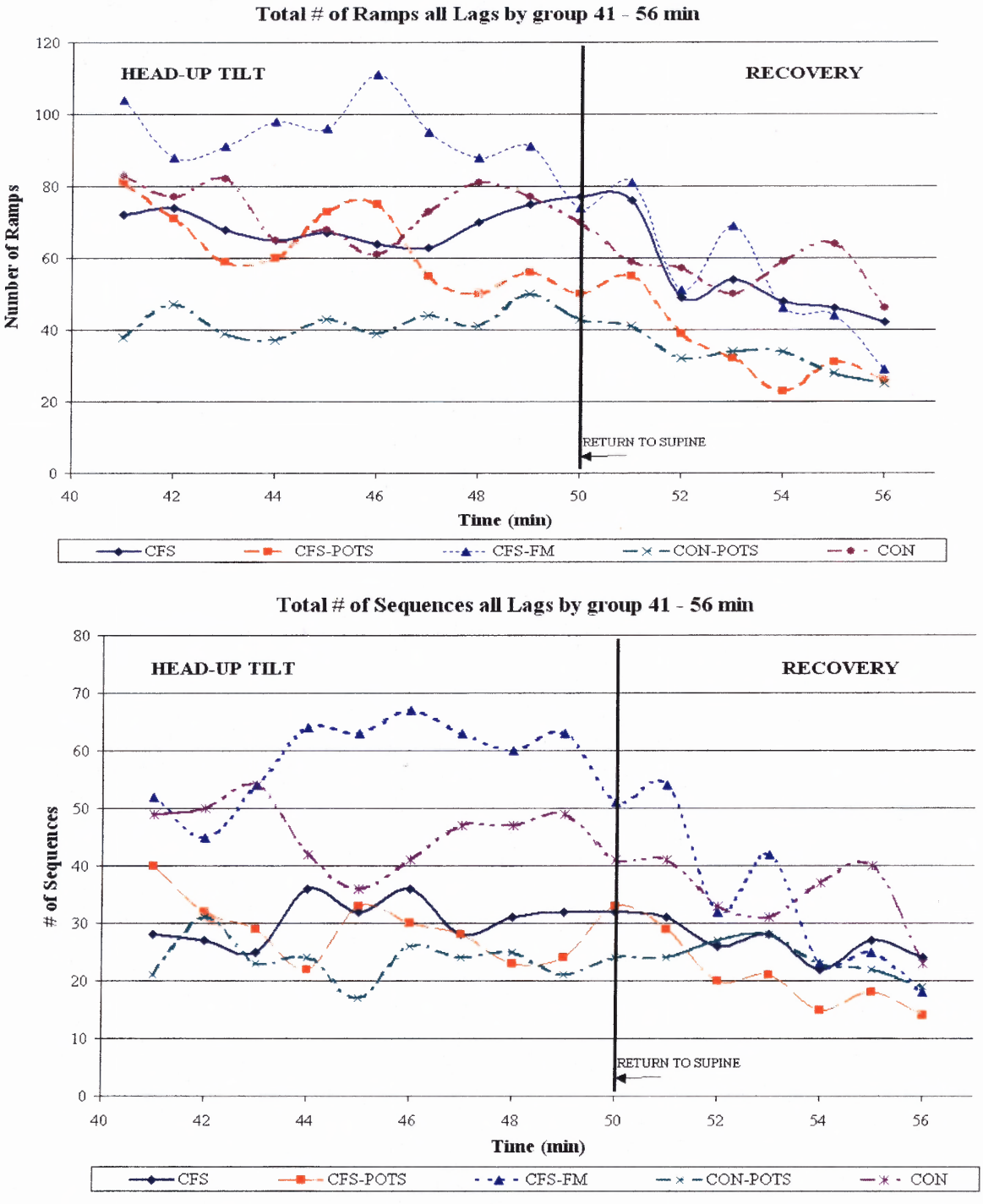


Figure C.5 Ramps and sequences for 41 – 56 minutes of HUT.

Figure C.6 are graphs of BEI for the beginning, (1-15 min) and middle (15-41 min) of HUT. The same trends seen in the group analysis of BRSI are evident in BEI.

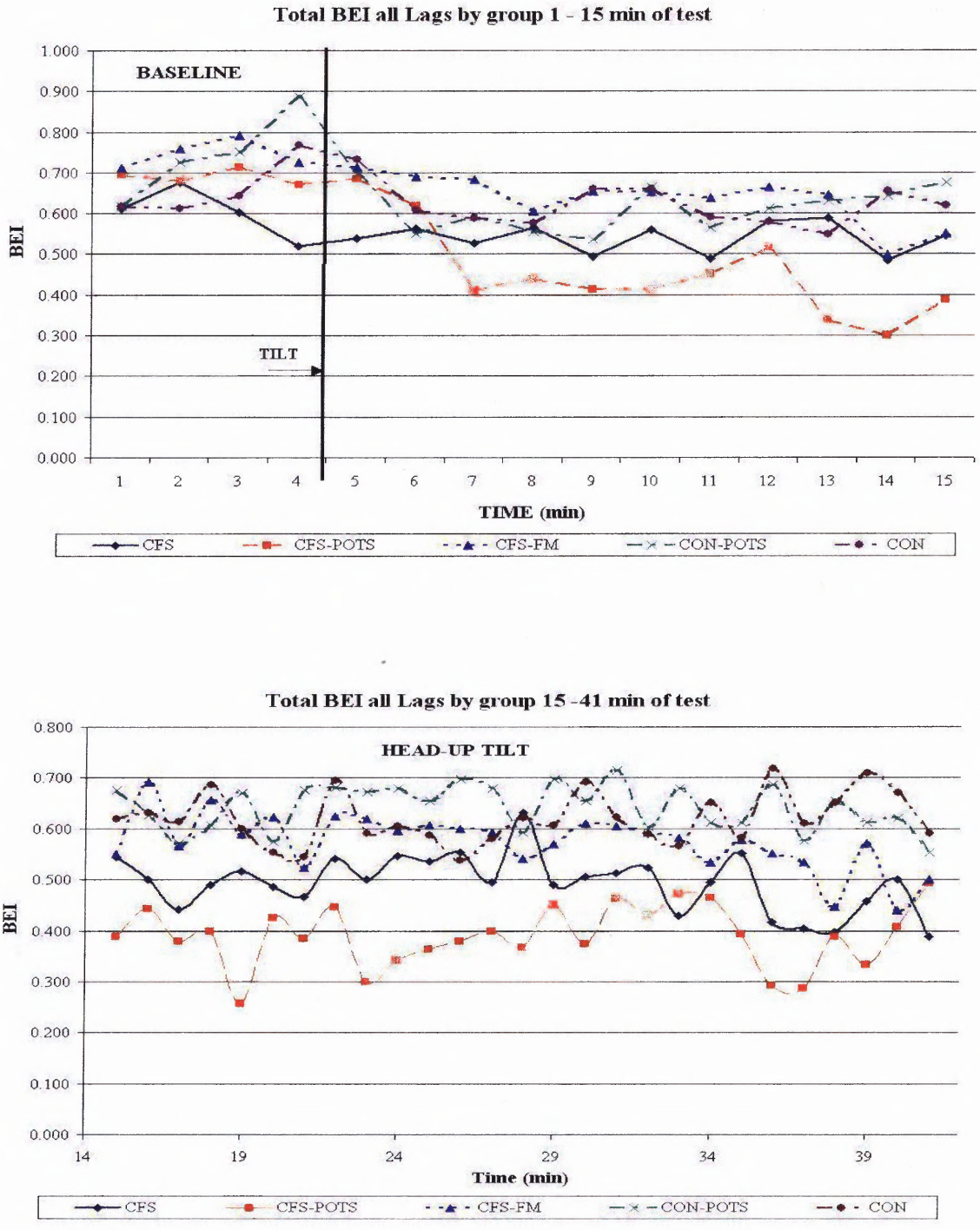


Figure C.6 BEI for first 41 minutes of HUT by group for each one-minute interval.

Figure C.7 is a graph of BEI for minutes 41 to 56 of HUT. BEI during recovery is not as prominent as it was when evaluating group BRSI results for the same time. Here CON-POTS has the highest increase when evaluating BEI. In the BRSI graph for the same time CFS-FM is by far the largest recovery spike. The similarities between CON

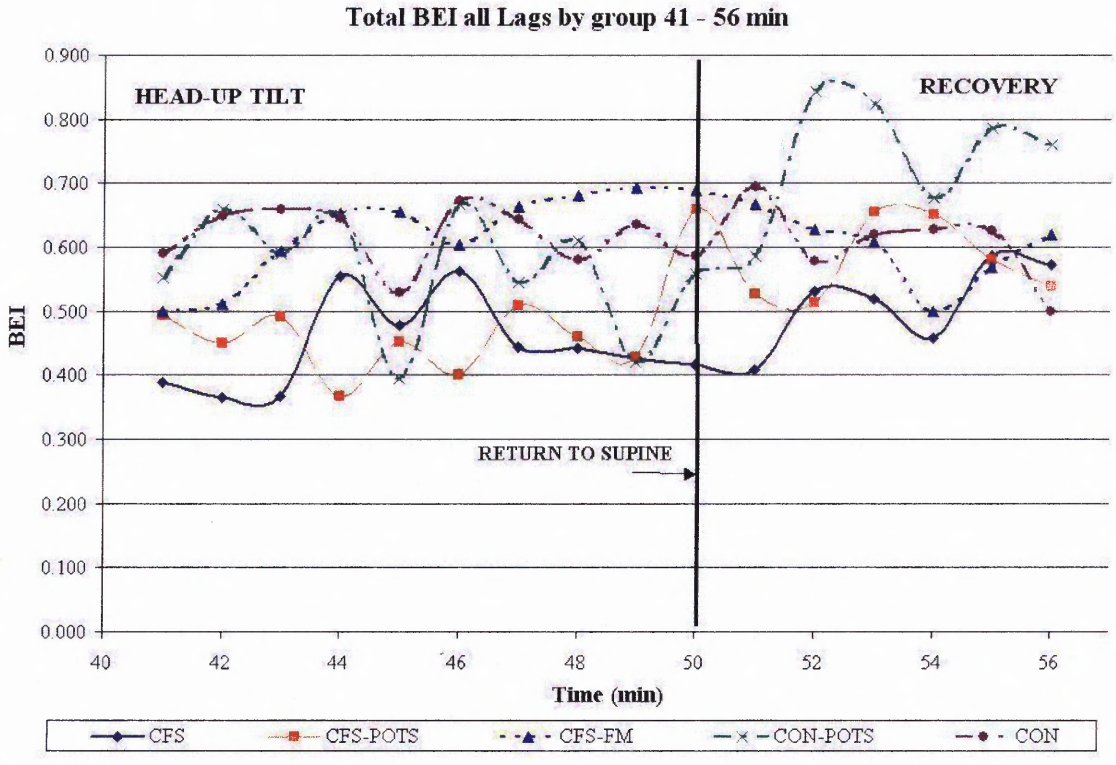


Figure C.7 BEI for the remaining 41 – 56 minutes of HUT.

and CFS seen in the BRSI graphs are not as clear when viewing BEI data. This is also true for the groups CON-POTS and CFS-POTS. BEI may or may not be indicating complimentary information as reported by Di Rienzo et al. [13], but the differences noted above are clear.

Figure C.8 show results using the alternative method of calculating an average BRSI as described in Section 3.1 are shown below.

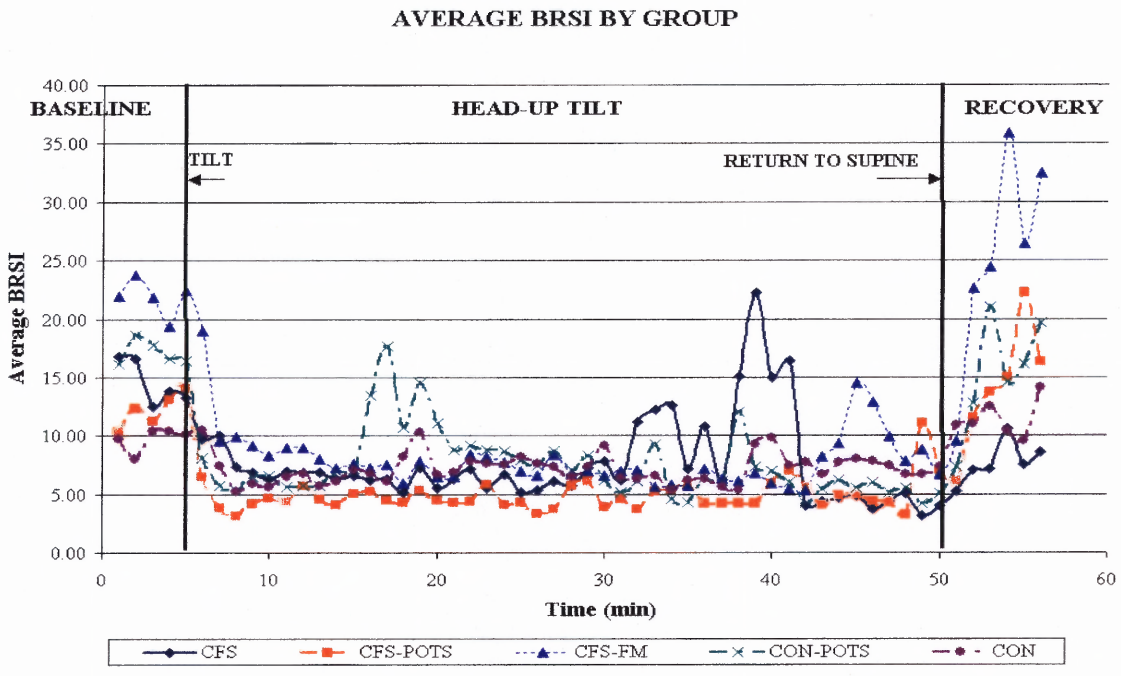


Figure C.8 Graph of average BRSI by group using alternate method of calculation.

A visual comparison between Figure C.8 and Figure 3.1 will show that the spikes present for groups CON-POTS, CFS and CFS-FM are higher in value than those illustrated in Figure 3.1. Although these are visually the most obvious differences between Figure 3.1 and the Average BRSI by Group graph, there are subtle differences through out the graph for all values.

The calculation of a true weighted average for BRSI is the more appropriate method of analysis and the procedure that was followed through out this thesis.

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